**ABSTRACT** BOOK

# SETAC NORTH AMERICA 43RD ANNUAL MEETING

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"BRIDGING INNOVATION AND SUSTAINABILITY"

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# Abstract Book

## SETAC North America 43rd Annual Meeting

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This book comprises the abstracts from the 43rd annual meeting of the Society of Environmental Toxicology and Chemistry – North America (SETAC North America), conducted from 13–17 November 2022 in Pittsburgh, PA, USA, and online.

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# About SETAC

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, analysis and solution of environmental problems, the management and regulation of natural resources, environmental education, and research and development.

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

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- Publish scientific journals, a newsletter and special technical publications
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For further information, contact one of our offices:


**SETAC Africa**
**SETAC Europe**
Avenue des Arts, 53
B-1000 Brussels, Belgium
**T** +32 2 772 72 81
**E** setaceu@setac.org

**SETAC Latin America**
**SETAC North America**
PO Box 12804
Pensacola, Florida, 32591-2804 USA
**T** +1 850 469 1500
**E** setac@setac.org

**SETAC Asia-Pacific**
Avenue des Arts, 53
B-1000 Brussels, Belgium
**T** +32 2 772 72 81
**E** setaceu@setac.org


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## Track 1: Environmental Toxicology and Stress Response

### 1.01.P Adopting Alternative Approaches to Animal Testing for Ecotoxicity Assessments: Approaches and Avenues for the Future

### 1.01.P-Mo001 Incorporating Ecologically Relevant Behaviors, Age at Exposure, and Two Species in Assessing the Toxicity of Thiamethoxam in Fish

***Tisha King-Heiden***[1]*, Shayla Victoria*[2]*, Sara Duffy*[1]*, Megan Hein*[1] *and Elisabeth Harrahy*[3]*, (1) University of Wisconsin, La Crosse, (2) University of Mississippi, (3) University of Wisconsin, Whitewater*

Alterations in behavior can serve as a sensitive indicator of the ecological consequences of exposure to environmental contaminants at concentrations that do not elicit significant morphological or adverse health outcomes. And while early embryonic stages of development tend to be more sensitive, many standard toxicity assays begin exposure post-hatch. Thiamethoxam (TXM) has emerged as a contaminant of concern due to its frequent detection in aquatic ecosystems and pseudopersistence, posing potential risks to non-target species. Here we summarize our work evaluating the sublethal toxicity of TXM following chronic embryonic or larval exposure in two species of fish, zebrafish and fathead minnow. Fish were exposed to target concentrations ranging from 0.02 to 200 μg TXM/L beginning either post-fertilization (embryonic) or post-hatch (larval) for a total of 5 (zebrafish) or 8 (fathead minnow) days. In general, fathead minnows were more sensitive to TXM exposure than zebrafish. Embryonic exposure resulted in reductions in growth and hatching success, along with altered embryonic motor behaviors, and/or predatory escape responses while no adverse effects were seen following exposure post-hatch. Our work illustrates the need to integrate age at exposure, species sensitivities, and ecologically relevant behavioral endpoints in toxicity testing to better understand the potential for environmental contaminants to impair the ecological health of organisms.

### 1.01.P-Mo002 Investigation of Possible Cardio- and Neuro-Toxic Effects Incited by Parabens to Daphnia magna

***Kojo Eghan****, Sangwoo Lee and Woo Keun Kim, Korea Institute of Toxicology (KIT), Korea, Republic of (South)*

Parabens have estrogenic and anti-androgenic qualities, which means they can alter the hormonal control of energy metabolism, causing obesity and metabolic health problems, as well as cardiovascular and neurological problems. On the other hand, studies on the health effects of parabens are contradictory. There is presently inadequate proof of parabens' detrimental effects on aquatic species. Our goal is to see if four parabens (methyl, ethyl, butyl, and propyl parabens) have any negative effects on *Daphnia magna's* cardiovascular and neurological systems.

Physiological tests and gene transcription analyses were performed 48 hours after acute exposure to *Daphnia magna* at various concentrations.

The heart rates and thoracic limb activity of the exposed daphnids were inhibited in a time and dose-dependent manner. The butylparaben group had the highest heart rate inhibitions. Butylparaben once again demonstrated the strongest significant suppression of thoracic limb activity in the highest concentration group. Ethylparaben and propylparaben had no effect on gene transcription in the cardiomyopathy group. At least one gene in the GABAergic synapse and acetylcholine receptor groups was substantially affected by all parabens.

The upheaval in *Daphnia magna's* heart rate and limb activity produced by the four examined parabens is extremely considerable, according to our findings. Unexpectedly, the cardiomyopathy group had fewer genes that were significantly impacted. Exposure to parabens does not appear to have had a significant impact on the nervous system. More research is needed, and is now underway, to fully comprehend the complex mechanism by which the neurobehavioral system of *Daphnia magna* is impacted

**1.01.P-Mo003 Marine Effluent Toxicity Testing: Evaluation of Alternative Testing Methods for Assessing Metal Toxicity**

***Dalton Scott Allen**, Maddie Wiencek, Michaela Kelly and Marlo K. Sellin Jeffries, (1) Texas Christian University*

As a result of the rapid development of the industrial sector, discharge of heavy metals into aquatic environments has become an issue of environmental concern. Industrial effluents discharged into receiving environments must be assessed for potential toxicity using standardized testing methods that often feature fish; however, there is a growing demand to employ animal alternatives. Identification of alternative methods for assessing acute metal toxicity in freshwater receiving environments have proven to be a challenge. Specifically, the fish embryo toxicity (FET) is known to be less sensitive to metals and thus, less predictive than traditional toxicity testing methods that employ larval or adult fish. Whether this relationship between the FET test and larval toxicity tests persists when using marine fish has yet to be determined. Thus, the goal of this study was to compare the results of FET and larval growth and survival (LGS) tests conducted with Ni and V in two model species, the sheepshead minnow and inland silverside. To achieve this, FET and LGS tests were conducted and the resulting toxicity values (i.e., median lethal concentrations) were compared. The results of these comparisons will be presented and the implications for the application of marine FET tests to assess complex effluents containing metals will be discussed.

**1.01.P-Mo004 Utilizing High-Throughput Screening to Rank and Prioritize Thyroid-Active Chemicals**

***Stephanie Eytcheson**[1], Michael Hornung[2] and Sigmund Degitz[2], (1) Oak Ridge Institute for Science and Education (ORISE) participant at U.S. Environmental Protection Agency, (2) U.S. Environmental Protection Agency*

High-throughput screening (HTS) is an approach to rapidly test a large number of chemicals for bioactivity at a specific molecular target. The ultimate goal of HTS is to reduce the cost of generating data to support assessing the risks from chemical exposure. The US EPA's Toxicity Forecaster (ToxCast) has a library of HTS data which can be used to prioritize chemicals of concern. One gap in thyroid-related HTS assays includes the thyroid hormone carrier proteins transthyretin (TTR) and thyroxine-binding globulin (TBG). TTR and TBG maintain the levels of free versus bound thyroid hormone and serve as circulating hormone transport proteins to deliver thyroid hormone to target tissue. To address this gap, a fluorescent high-throughput assay has been developed to assess inhibition of TTR or TBG. 8-Anilino-1-naphthalenesulfonic acid ammonium salt (ANSA) is a probe which fluoresces when bound to TTR or TBG. In the assay, inhibitory chemicals displace ANSA from the protein resulting in a loss of fluorescence. A two-tiered approach is being utilized to rank and prioritize chemicals for further testing. Approximately 1800 chemicals from the ToxCast phase 1, phase 2, and e1k libraries have been screened for activity at a single high concentration. Chemicals with greater than 20% inhibition are considered inhibitory. The total number of inhibitory chemicals in each assay were 825 in the TBG assay and 1017 in the TTR assay. There were 166 and 355 chemicals with greater than 80% inhibition of TBG and TTR, respectively. Chemicals with greater than 80% inhibition will be moved on to concentration response testing to define the $IC_{50}$. Results from the assays will allow for ranking and prioritization of chemicals to be tested *in vivo* and will aid in the development of a framework to predict *in vivo* effects from *in vitro* HTS data. *The contents of this abstract neither constitute, nor necessarily reflect, US EPA policy.*

**1.01.P-Mo005 Comparative Cytotoxicity of Seven Per- and Polyfluoroalkyl Substances (PFAS) in Six Human Cell Lines**

***Megan Solan**, Sanjanaa Senthilkumar, Grace V. Aquino, Erica Bruce and Ramon Lavado, Baylor University*

Human exposures to perfluoroalkyl and polyfluoroalkyl substances (PFAS) have been linked to several diseases associated with adverse health outcomes. Animal studies have been conducted to study these outcomes, though these may not be sufficient due to the inherent differences in toxicokinetics between humans and rodents. Acquiring relevant data on the health effects of short-chain PFAS can be achieved through high-throughput methods supported by *in vitro* human cell-based models. Specifically, cytotoxicity assays are the crucial first

step to providing meaningful information for *in vitro* methods used for determining safety and providing baseline information for further testing. In this study, we exposed human cell lines representative of six different tissue types, including colon (Caco-2), liver (HepaRG), kidney (HEK293), brain (HMC-3), lung (MRC-5), and muscle (RMS-13) to five short-chain PFAS [undecafluoro-2-methyl-3-oxahexanoic acid (HFPO-DA), perfluorobutanesulfonic acid (PFBS), perfluorohexanoic acid (PFHxA), perfluorohexanesulfonic acid (PFHxS), and 6:2 fluorotelomer alcohol (6:2 FTOH)] and two legacy PFAS [perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic (PFOS)]. Exposure the individual PFAS were assessed using a range of eight concentrations, starting from a low concentration (10 pM) to a high concentration of (100 μM). Our results indicated that Caco-2 and HEK293 cells were the least sensitive to PFAS exposure, while HMC-3, HepaRG, MRC-5, and RMS-13 demonstrated decreases in viability in a relatively narrow range (~1-70 μM). Our data suggest that PFAS do not exert toxicity on all cell types equally and the cytotoxicity estimates we obtained varied from previously reported values most likely due to differences in cell line sensitivity. Overall, this study is novel because it uses human cell lines that have not been widely used to understand human health outcomes associated with PFAS exposure.

**1.01.P-Mo007 Use of Primary Rainbow Trout Hepatocytes to Determine In Vitro Intrinsic Clearance for Bioaccumulation Assessment in Fish**

*Anett Ullrich[1], Tanja Krimmling[1], Melissa Thiede[1], Heike Laue[2], Lu Hostettler[2] and* ***Dieter Runge****[1], (1) Primacyt Cell Culture Technology GmbH, Germany, (2) Givaudan Schweiz AG, Switzerland*

In order to improve *in silico* predictions of chemical bioaccumulation in fish, methods are needed to estimate hepatic biotransformation and incorporate this information into established computational models. One promising approach involves the measurement of intrinsic clearance using *in vitro* biotransformation systems derived from liver tissue. The OECD Test Guidelines (TG) 319A and B support this approach and therefore contribute to alternative methods to animal experiments in context of the 3Rs.

Primary Rainbow trout hepatocytes (*Oncorhynchus mykiss*) were isolated from animals kept under controlled environmental conditions and cryopreserved. Cells remained a high viability after thawing over a time frame of at least 2-3 years and kept viabilities above 90 % under shaking conditions (1000 rpm, 14 °C) for metabolism and depletion assays in suspension cultures. The metabolites of three main Cytochrome P450 reactions as well as glucuronidation and sulfation reactions were quantified.

Furthermore, the *in vitro* intrinsic clearance was determined with the reference chemical Pyrene (0.025 μM) using the cells in substrate depletion assays according to the OECD TG 319 A with direct comparison to heat-inactivated hepatocytes as negative control. Incubations were performed with $2*10^6$ viable cells/mL for up to 15 minutes at 11 °C.  Pyrene concentration was analyzed by GC-MS with anthracene as internal standard for quantification. The measured *in vitro* intrinsic clearance values ($CL,_{IN\ VITRO,int}$ of 2.78-5.41 $mL*h^{-1}*10^6$ $cells^{-1}$) were  comparable to results from the international ring trial. These $CL,_{IN\ VITRO,int}$ can be used to extrapolate to the whole body biotransformation rate to estimate a BCF (bioconcentration factor) using *in vitro* to *in vivo* extrapolation models or as direct inputs in physiologically based toxicokinetic models (PBTK) for fish bioaccumulation assessment.

Primary hepatocytes from Rainbow trout and other fish species, e. g. Atlantic salmon or Common carp, as well as from other species like chicken or duck, are becoming increasingly important *in vitro* models as alternatives to animal testing in the field of environmental toxicology, in particular for bioaccumulation assessment.

**1.01.P-Mo008 Physiological and Transcriptomic Effects of Hexafluoropropylene Oxide Dimer Acid in Caenorhabditis elegans During Development**

***Zuying Feng****[1], Flannery McLamb[1], Jeanne P. Vu[1], Sylvia Gong[1], Richard M. Gersberg[2] and Goran Bozinovic[3], (1) Boz Life Science Research and Teaching Institute, (2) San Diego State University, (3) University of*

*California, San Diego*
Hexafluoropropylene oxide dimer acid (HFPO-DA) is one of the emerging per- and polyfluoroalkyl substances (PFAS) and was developed to phase out the toxic and bioaccumulative perfluorooctanoic acid. Compared to legacy PFAS, HFPO-DA has not been extensively studied, and mechanistic explanation of exposure effects is lacking. To evaluate the potential toxicity of HFPO-DA with a cost- and time-efficient approach, we exposed *C. elegans* larvae to $4\times10^{-9}$–4 g/L HFPO-DA in liquid media and performed assays measuring developmental, behavioral, locomotor, and transcriptional effects at various exposure levels. After 48 hours of 1.5–4 g/L HFPO-DA exposure, acute developmental toxicity was observed as developmental delay; statistically significantly delayed ($p < 0.05$) progeny production was observed in worms exposed to 2–4 g/L HFPO-DA. After 48 hours of $4\times10^{-9}$–0.4 g/L exposure, no significant behavioral or locomotor effect was observed relative to the 0 g/L control group. Statistically significant differential gene expression was identified with over 99% confidence via the R-package NOISeq in all fourteen 48-hour $1.25\times10^{-5}$–4 g/L HFPO-DA exposure groups, except for $6.25\times10^{-5}$ g/L. Among 10298 genes analyzed, 2624 differentially expressed genes (DEGs) were identified in the developmentally delayed 4 g/L group only, and 78 genes were differentially expressed in at least one of the thirteen exposure groups testing $1.25\times10^{-5}$–2 g/L HFPO-DA exposures. Genes encoding for detoxification proteins such as cytochrome P450 enzymes and UDP glucuronosyltransferases are upregulated in 0.25–4 g/L acute exposure groups. In the lower exposure concentration groups, statistically significant gene expression changes were also observed, though these DEGs did not share any biological functions, except for six ribosomal protein-coding genes. While our transcriptional data is insufficient for conclusive mechanistic explanation of HFPO-DA exposure effects, we report consistent statistically significant gene expression differences detected in low concentration exposure groups, including the lowest concentration tested for transcriptional changes, $1.25\times10^{-5}$ g/L. This concentration is also only one magnitude higher than the highest environmentally detected level. By combining *C. elegans* with cost- and time-efficient assays, our toxicity screening covering both acute and low concentrations informs future targeted analyses that focus on low-dose HFPO-DA exposure effects.

**1.01.P-Mo010 Using a Human Immune Cell-Based Bioindicator System to Examine the Effects of Raw and Treated Oil Sands Process Affected Water**
***Sunanda Paul**, Dustin M.E. Lillico and James L. Stafford, University of Alberta, Canada*
Industry scale mining of the Alberta oil sands bitumen deposits produces large volumes of oil sand process affected water (OSPW) as a by-product. OSPW is a complex mixture of organic and inorganic components which has been reported to have toxic effects on aquatic and terrestrial organisms. Consequently, OSPW cannot be released into the environment without some form of treatment designed to remove or neutralize its toxic effects. Various OSPW remediation strategies have been investigated with the goal of removing toxic constituents, which includes Advanced Oxidation Processes (AOPs) that in general degrade specific organic components within OSPW, including the well-established toxic naphthenic acid (NA) species. The toxicity of AOP-treated OSPW needs to be thoroughly tested to evaluate the efficacy of the remediation procedures. Our lab has been working to develop an *in vitro* immune cell-based bioassay system to study the immunomodulatory effects of OSPW. In the present study, THP-1-cells, a human macrophage-like cell line, were exposed to sub-lethal doses of raw and AOP-treated OSPW to evaluate their effects on the expression of immune genes, cell-surface proteins, and cytokine secretion levels using quantitative(q)-PCR, flow cytometry and a human cytokine array. Results showed that raw OSPW exposures for as short as 6 hours significantly induced a robust proinflammatory cytokine gene expression profile. Cells exposed to raw OSPW for 24 hours also showed elevated secretion levels of the cytokines tumor necrosis factor (TNF)-α, interleukin (IL)-1ß, IL-6, and IL-8, which did not occur in the control treatment group. Furthermore, raw OSPW exposures increased the surface expression levels of the biomarkers CD40 and CD54 revealing that raw OSPW contains potent immunostimulatory compounds that activate human macrophages. In comparison, AOP-treatment of OSPW significantly abrogated its immunostimulatory effects indicating that AOPs remove inflammatory components from raw OSPW, an effect that correlated with reduction of NA concentrations in the treated waters. Finally,

transcriptomic analysis demonstrated the robust inflammatory effects of raw OSPW exposures on human macrophages and supports that AOP effectively removes these compounds. Overall, our study indicates that THP1-cells serve as a useful bioindicator system to examine the immunotoxicity of OSPW and can be used to evaluate the effectiveness of OSPW remediation strategies.

### 1.01.P-Mo011 Designing a Physiologically Based Toxicokinetic (PBTK) Model for Fish Species in Arctic Environments

***Gregory Martin Langlois***[1]*, Knut Erik Tollefsen*[2]*, Ian Edhlund*[1] *and Peter van den Hurk*[1]*, (1) Clemson University, (2) Norwegian Institute for Water Research (NIVA), Norway*

To model a complete Source To Outcome Pathway (STOP) for arctic species, it is essential to parameterize the potential environmental concentrations and the pathways that link outside environment concentrations to internal target site concentrations so that an Adverse Outcome Pathway (AOP) can be modeled starting with the Molecular Initiating Event based on target site concentrations. To come to this Aggregate Exposure Pathway model, physiologically based toxicokinetic (PBTK) models can be applied to predict bioconcentration and internal distribution of various chemicals in different species. PBTK models consist of a variety of basic equations that describe the absorption, distribution, metabolism, and excretion of a compound in the body. These equations are linked in a series of ordinary differential equations and parameterized by the physiology (e.g.blood flow to organs, tissue volumes, effective respiratory volume, etc.) of the species being investigated. The majority of PBTK models for fish have been described for freshwater species such as rainbow trout, from temperate waters, but few or no models currently exist for Arctic species. Furthermore, models to predict the physiological and metabolic parameters for fish are often derived solely from one species, and realistic extrapolation methods for other species, other habitats (freshwater vs. sea water) and climatic regions (tropical vs. terrestrial vs. Arctic) are urgently needed. This specific work aims to fill PBTK modeling gaps for arctic marine fish species, using halibut (Hippoglossushippoglossus) as a model species, by exploring their physiology and possible effects of changing environmental conditions on internal pollutant concentrations at the site of molecular action (molecular target site). This work will help answer questions about how changing conditions due to climate change, such as water temperatures and ocean salinity, will impact model parameters (e.g. metabolism, respiration), and in turn, affect bioconcentration and potential adverse outcomes to humans and environmental species.

### 1.01.P-Mo012 Addressing Ionization Improves Performance of In Silico Models for Predicting the Bioconcentration Factor (BCF)

***Geetesh Devineni****, Chaitrali Patil, Adelina Voutchkova-Kostal and Jakub Kostal, The George Washington University*

Experimental measurement of the Bioconcentration Factor (BCF), a key metric of chemical accumulation in living organisms, is time-consuming, expensive and highly variable, depending on the method and organism. As a result, many Quantitative Structure Activity Relationships (QSARs) have been developed to provide rapid and low-cost in silico prediction of BCF. However, no single model adequately captures the complex nonlinear relationship between BCF and physiochemical properties, such as octanol-water distribution coefficient (Log P). Herein we show that by modeling physicochemical properties, which we capture explicitly compared to existing methods, of all relevant charged species at biological pH we can develop a model that improves prediction of BCF for many chemical classes, including persistent, bioaccumulative, and toxic (PBT) substances such as polychlorinated biphenyls (PCBs). We postulate that explicit consideration of ionization is likely to improve models for other properties and activities related to ecotoxicity and environmental fate of commercial chemicals, thus furthering our goal of developing robust alternative approaches that alleviate animal testing.

**1.01.P-Mo013 Predicting Fish Acute Toxicity Using a Bayesian Weight of Evidence Approach**

***Michelle Rau Embry***[1], *Kristin A. Connors*[2], *Jannicke Moe*[3], *Anders Madsen*[4], *Thomas Braunbeck*[5], *Kristin Schirmer*[6], *Stefan Scholz*[7], *Raoul Wolf*[8] *and Adam Lillicrap*[3], *(1) Health and Environmental Sciences Institute (HESI), (2) Procter & Gamble, (3) Norwegian Institute for Water Research (NIVA), Norway, (4) Hugin Expert, Denmark, (5) University of Heidelberg, Germany, (6) Utox, Eawag - Swiss Federal Institute of Aquatic Science and Technology, Switzerland, (7) Helmholtz Centre for Environmental Research – UFZ, Germany, (8) Norwegian Geotechnical Institute (NGI), Norway,*

The acute fish toxicity test (AFT; OECD TG 203) is currently required for chemical hazard and risk assessment purposes in many different legislations and regulations. The fish embryo toxicity test (FET; OECD TG 236) has been proposed as an alternative to using juvenile fish to reduce the number of live animals required for hazard and risk assessments of chemicals. However, FET data are not yet accepted as a replacement for AFT data for regulatory purposes such as REACH. It was suggested that the development of a Weight of Evidence (WoE) approach would help increase confidence in the use of FET data to predict acute fish toxicity for regulatory applications. A multi-sector project, "Strengthening Weight of evidence for FET data to replace Acute Fish Toxicity (SWiFT)" developed a Bayesian Network (BN) model to provide a quantitative approach to integrate various lines of evidence in a probabilistic manner. A BN is a probabilistic graphical model that represents a set of variables and their conditional dependencies via a directed acyclic graph. The purpose of the proposed acute fish toxicity BN model is to integrate data from large ecotoxicological and physico-chemical datasets and apply it in a WoE context to predict fish acute toxicity of chemicals using data on fish embryo toxicity tests in combination with other types of information. The additional lines of evidence include physical-chemical properties, fish gill cell-line cytotoxicity assay, neurotoxicity, biotransformation predictions, toxicity data for other taxa (e.g., invertebrates and algae), read across, and *in silico* predictions. The output is comprised of probabilities of toxicity intervals for the chemical in question. The BN model is freely available and model performance was assessed by running the BN model with input data from a select chemicals with complete datasets and comparing the outcome, i.e., the predicted acute toxicity of selected chemical substances to juvenile fish, to the observed toxicity of the same substances. This presentation will focus on model interpretation and how the results can be used as a WoE strategy for predicting fish acute toxicity and facilitating the use of FET data.

**1.01.P-Mo014 Application of Biomimetic Extraction to Measure Toxicity Reduction in Oil Sands Process-Affected Water After Wetland Treatment**

***Julia Brueggeman***, *Frank Gobas and Alexander Cancelli, Simon Fraser University, Canada*

Accumulation of oil sands process-affected water (OSPW) in Alberta's Oil Sands Region poses an environmental threat. As a result of Alberta's zero-discharge policy, OSPW is stored on site. One potential treatment solution being tested at the pilot scale is a constructed treatment wetland system (CWTS). This study aims to assess the treatment efficacy of Imperial Oil's Kearl treatment wetland using passive sampling to measure changes in OSPW toxicity as it flows through the wetland. Biomimetic extraction (BE) using solid-phase microextraction (SPME) fibers measures freely dissolved concentrations of the acid-extractable organic (AEO) fraction of OSPW and gives insight into its toxicity of OSPW through calibration with in-vivo toxicity metrics in chronic toxicity tests. This passive sampling method has the potential to replace traditional toxicity testing for whole effluent screening. The manual BE-SPME method was applied to aqueous samples taken at five points during the wetland flow-through process (30 days) and analysed using gas chromatography-flame ionization detection (GC-FID) to determine OSPW treatment efficiency and changes in toxicity due to wetland treatment. Toxicity testing with walleye (*Sander vitreus*) and an aquatic invertebrate (*Ceriodaphnia dubia)* is in progress. The results of this study, including toxicity testing, will be discussed.

**1.01.P-Mo015 Omics Data in Regulatory Toxicology: An Opportunity for Collaboration between Regulatory Risk Assessors and Researchers**

***Jone Corrales***[1], *Kara Koehrn, Adam Biales, David Bencic, Weichun Huang, Emily Vebrosky Nolan and Kellie*

*A. Fay, U.S. Environmental Protection Agency*
The Environmental Protection Agency's (EPA) New Approach Methods (NAMs) Work Plan was created to increase the rigor and sophistication of Agency assessments while reducing the reliance on vertebrate animal testing. The Toxic Substances Control Act (TSCA) requires EPA to meet the scientific standards for best available science and where practicable to reduce and replace the use of vertebrate animals in the testing of chemical substances by taking into consideration bioinformatics and high-throughput screening methods and the prediction models of those methods. To this end, the characterization and interpretation of chemical-driven changes in gene expression, e.g., transcriptomic data, is a potentially powerful NAM that can inform chemical risk assessment. Given the difficulty of the application of omics data to risk assessment due to the complexity of data interpretation and use in identifying endpoints of concern, researchers and regulators need to work together. The goal of this project is three-fold: (1) to maintain a collaborative effort between EPA researchers and regulatory scientists on the use of transcriptomics data in risk assessment, (2) to consider the relevance and reliability of omics data for use in TSCA risk evaluations, and (3) to support the use of the best available science in TSCA risk evaluations. For this work, scientists in EPA's Office of Research and Development (ORD) with expertise in transcriptomics and scientists in the Office of Pollution Prevention and Toxics (OPPT) with experience in risk assessment are working together to apply the use of transcriptomics data from fathead minnow embryos exposed for 24-h to three TSCA high priority substances: butyl benzyl phthalate, dibutyl phthalate, and diisobutyl phthalate. The data will be used to derive transcriptomic points of departure (tPODs), inform mode of action, and support chemical read-across. This case study will examine the application of omics data for decision making under TSCA. Moreover, this work supports the growing global interests on applying omics data in risk assessment; for example, in June 2021, the Organization for Economic Cooperation and Development (OECD) held a session to discuss how the development of omics frameworks will increase the confidence on applying omics data in risk assessments. Disclaimer: The views expressed in this poster are solely those of the authors and do not represent the policies of the U.S. EPA.

**1.01.P-Mo016 Comparison of In Vitro to In Vivo Data Generated in Bioaccumulation Studies**
***Emma Danby***[1] *and Lindsay Knight*[1,2]*, (1) Labcorp Early Development Laboratories Limited, United Kingdom, (2) Metabolism, Labcorp, United Kingdom*
Fish bioconcentration or biomagnification studies are carried out to determine whether substances have the potential to bioaccumulate and have potential to biomagnify throughout different trophic levels. The concentration of test material accumulated in the fish over time is compared to that in the surrounding media or the diet and the rate of uptake and depuration are calculated. There are increasing requirements to conduct these studies on a broad variety of industrial chemicals, pharmaceuticals and crop protection products across the globe, resulting in the use of significant numbers of fish.The preferred method of testing requires three doses of chemical (including a control) with up to 100 fish per dose. However, following the revision to the OECD Test Guideline (OECD 305: 2012), there is now the option to test on one concentration and the control only, providing there is scientific justification. This has the potential to decrease the numbers of fish used in these tests by one third (100 fish per study). The best way to reduce animal numbers further is to replace animals altogether with validated *in vitro* alternative studies such as the OECD 319 study (Determination of *in vitro* intrinsic clearance using cryopreserved rainbow trout hepatocytes or S9 fractions). The liver intrinsic clearance is used directly as an input to physiologically based toxicokinetic (PBTK) models for fish bioaccumulation. Alternatively, this value may be extrapolated to a whole-body (*in vivo*) biotransformation rate constant using an appropriate *in vitro* to *in vivo* extrapolation (IVIVE) model. The *in vivo* biotransformation rate can be included into *in silico* models for prediction of bioconcentration factors (BCF).

Here we present our preliminary results comparing the in vivo and in vitro studies side by side on the same chemical substances

**1.01.P-Mo017 Anti-Obesity Activities of Natural Dietary Products (Cannabidiol, Indole-3-Carbinol and Trans-Resveratrol) in the Human Liver Cell Line hepaRG and Human Adipocytes**

***Sanjanaa Senthilkumar****, Megan Solan and Ramon Lavado, Baylor University*

An increasing number of diseases related to obesity are an alarming problem worldwide. Therefore, new therapeutic methods are constantly sought to prevent, treat, and alleviate symptoms of the diseases associated to obesity. This study investigates the effects of three natural compounds (indole-3-carbinol, I3C, a bioactive indolic compound found in cruciferous vegetables; cannabidiol, CBD, the active ingredient derived from the hemp plant; and trans-resveratrol, TRV, a natural compound present in grapes, red wine, and berries) on the fatty acid accumulation in the human liver cell line HepaRG, a well-established model for non-alcoholic fatty liver disease (NAFLD) and in human pre-adipocytes (adipose-derived mesenchymal stem cells, MSC). LC50s of each compound were in the high µM range (approximately 30 mg/L), showing the low toxicity of these compounds. Determination of the selected compounds in cell media showed no significant differences during the time of exposure, suggesting that no significant metabolism or degradation happened during the exposure time. Quantification of the bioaccumulation of lipid droplets on exposed HepaRG revealed significant lipolysis and prevention of fatty acid accumulation when exposed to 1 nM of I3C and 100 nM of CBD. Same effect was observed when exposed to TRV, but at cytotoxic concentrations (higher than 10 µM). On MSC cells a significant inhibition of lipogenesis and adipocyte differentiation was observed in cells exposed to 0.1 nM of I3C and to 1 nM of CBD, while no significant effect was observed with TRV. This study provides a significant contribution to advancing the understanding of preventative dietary strategies that target adipocyte differentiation and NAFLD.

**1.01.P-Mo018 Reduced Transcriptomics to Characterize the Ecotoxicity of 17a-Ethinylestradiol in Amphibian, Bird and Fish Embryos: An EcoToxChip Case Study**

***Markus Hecker***[1]*, Alper James Alcaraz*[1]*, Emily Boulanger*[2]*, Jianguo Xia*[2]*, Jessica Ewald*[2]*, Jessica Head*[2]*, Doug Crump*[3]*, Nil Basu*[2] *and Natacha S. Hogan*[1]*, (1) University of Saskatchewan, Canada, (2) McGill University, Canada, (3) Environment and Climate Change Canada, Canada*

The development of new approach methods (NAMs) that aim to reduce the use of live animals in ecotoxicity testing is urgently needed to meet current regulatory mandates to assess the toxicological risks of the vast and rapidly increasing number of chemicals used by society. However, most NAMs are limited in that they focus on certain model species or specific taxonomic groups. This study aimed to apply a novel standardized reduced transcriptome assay (EcoToxChip system) using short-term embryonic exposures to compare molecular response patterns across model species representing three vertebrate taxa (amphibian, bird, fish). Animals were exposed to 17α-ethinylestradiol (EE2), representing a well-described model xenoestrogen. Embryos of African clawed frog (XL), fathead minnow (FHM) and Japanese quail (JQ) were exposed to graded concentrations of EE2 for 4-9 days, prior to independent feeding (XL/FHM) or pre-hatch (JQ). Embryos were then processed for molecular analyses using the EcoToxChip. Reduced transcriptome data was analyzed using EcoToxXplorer.ca, an intuitive online bioinformatics tool. A subset of the animals was then grown out for several weeks to assess apical and histological outcomes to anchor molecular responses. EcoToxChip analysis revealed 62, 69 and 57 differentially expressed genes in XL, JQ and FHM. In JQ and FHM, the majority of functional responses were associated with estrogen signaling and lipid/energy metabolism while XL revealed a slightly different response pattern with metabolic process being the main target. These effects are in accordance with histological alterations (e.g. massive accumulation of proteinaceous fluid in birds and fish) and altered growth probably due to energetic costs associated with protein synthesis. In conclusion, this study suggests that reduced transcriptome profiling after short-term embryonic exposures can be used to identify later but specific biological outcomes across diverse vertebrate taxonomic groups. This study is part of the EcoToxChip project (www.ecotoxchip.ca).

**1.01.V Adopting Alternative Approaches to Animal Testing for Ecotoxicity Assessments: Approaches and Avenues for the Future**

**1.01.V-01 Transcriptomic Dose-Response Analysis in Zebrafish Embryos to Estimate Estrogenicity and Long Term Aquatic Toxicity of Bisphenol A and Two of its Replacement Compounds**

***Phuoc Tyler Nguyen***[1]*, Jory Curry*[2]*, Jan Mennigen*[2]*, Florence Page-Lariviere*[1]*, John Prindiville*[1]*, Doug Crump*[1] *and Jason O'Brien*[1]*, (1) Environment and Climate Change Canada, (2)University of Ottawa, Canada*

For many endocrine disrupting compounds (EDCs), acute exposure studies may not reveal their long-term impact. However, long-term exposure studies have challenges including ethical concerns, prohibitive costs, and large sample size requirements making them unfeasible for routine testing and risk assessment. Recent research has shown that modelling changes in gene expression (i.e., transcriptomic dose response modelling [TDRM]) following acute exposure can be used to estimate doses that lead to chronic toxicity. In the present study, we exposed zebrafish embryos to a 5-point, 10-fold dilutions series of bisphenol A (BPA), two replacement compounds (bisphenol AF [BPAF] and Phenol, 4,4'-Sulfonylbis[2-(prop-2-en-1-yl)phenol] [TGSH]), and two estrogenic positive controls (diethylstilbestrol and ethinylestradiol) to determine effects on lethality, deformities, behaviour, energy expenditure, and gene expression. Embryos were exposed using a daily renewal regimen from 0 to 120 hours post fertilization (hpf). At 120 hpf we conducted a light-dark swim behaviour assay, measured energy consumption with an Alamar Blue assay, measured gene expression using QIAseq UPX 3' sequencing, and conducted a dose-response analysis of all measured endpoints. We found no effects on any morphological, behavioural or energy expenditure endpoints. Sequencing data is currently pending and will be used to calculate a transcriptomic point of departure for each chemical. These results will help elucidate the effectiveness of TDRM as a tool to provide quantitative mechanistic information on compounds, like suspected EDCs, that have limited toxicology data during risk assessment.

**1.01.V-02 Technical Framework for High Quality New Approach Methodologies (NAMs)**

***Elijah Petersen***[1]*, John Elliott*[1]*, John Gordon*[2]*, Nicole Kleinstreuer*[3]*, Emily Reinke*[4]*, Matthias Rösslein*[5] *and Blaza Toman*[1]*, (1) National Institute of Standards and Technology (NIST), (2) U.S. Consumer Product Safety Commission, (3) NICEATM, (4) U.S. Army Public Health Center, (5) Empa – Swiss Federal Laboratories for Material Science and Technology, Switzerland*

New approach methodologies (NAMs) are *in vitro*, *in chemico*, and computational approaches that can potentially be used to reduce animal testing. For NAMs that require laboratory experiments, it is critical that they provide consistent and reliable results. While guidance has been provided on improving the reproducibility of NAMs that require laboratory experiments, there is not yet an overarching technical framework that details how to add measurement quality features into a protocol. In this manuscript, we discuss such a framework and provide a step-by step process describing how to refine a protocol using basic quality tools: cause-and-effect analysis, flowcharts, check sheets, control charts, histograms, and scatterplots. The steps in this framework include 1) conceptual evaluation of the assay, 2) within laboratory evaluation, 3) statistical data analysis and reporting, and 4) interlaboratory testing (if needed). While each of these steps has discrete components, they are all inter-related and insights from any step can influence the others. Following the steps in this framework can help reveal the advantages and limitations of different design choices. Overall, the use of this technical framework can support the development of robust NAMs that can meet research and regulatory needs.

**1.01.V-03 Zebrafish Models as an Integrated Approach to Testing and Assessment (IATA) to Maximize Human and Environmental Health Protection While Reducing Animal Testing in Chemical Risk Assessment (CRA)**

*Lee Ellis*[1]*, John Achenbach*[1]*, Michael Morash*[1]*, Cindy Woodland*[2] *and* ***Roberta Moore***[2]*, (1) National Research Council of Canada, (2) Health Canada*

In 2018, the Government of Canada began researching the potential of the zebrafish (ZF) embryo and larvae as whole organism models to evaluate endocrine disruption beyond developmental effects (e.g., obesogenicity, adrenal perturbations) and as an alternative to the 28-day rodent assay for general toxicity assessments, thereby facilitating the 3Rs of reduction, refinement and replacement of animals in toxicity testing. In 2021, the Canadian government announced the intention to ban animal testing by 2035. Subsequently the platform is being developed as a dual application model for both human health and environmental risk assessment. Key to this research has been the incorporation of novel behavioral and transcriptomic platforms as early biomarkers of toxicity testing, along with the assessment of the absorption and metabolism of the test compounds. The ZF larval toxicity testing models used for this research project were designed at the NRC Canada and are based on the OECD fish embryo toxicity (FET) assay and the NRC general and behavioural toxicity (GBT) assay. Integral to a robust CRA is the use of data representing high biological coverage that results from a systemic model as a complement to cell-based assays in an IATA. Through a tiered approach, data generated from the relevant ZF models, such as the results from gene expression testing could be considered as a first tier of testing. The results could subsequently be used to select appropriate human or animal cell-based assays to be used for higher tier testing. This IATA approach would then become valuable for both human health and environmental risk assessment. In order to further develop zebrafish testing for the purpose of environmental assessment, the testing must be conducted beyond the larval stages. The additional use of juvenile and adult zebrafish allows for the evaluation of the effects of chronic exposure to environmentally relevant concentrations of chemicals. These full life cycle models for ecotoxicity assessment include an expanded set of behavioural tests along with measures of population-level effects such as changes in fecundity and fertility. The ZF model has proven to be a valuable platform for toxicity testing and is promising to be a fundamental part of IATAs for both environmental and human health risk assessment. Importantly, international collaborations are under way in order to validate the ZF model as a globally harmonized regulatory new approach methodology (NAM) for CRA.

**1.01.V-04 Locomotor Ability and Brain Gene Expression in Drosophila melanogaster Exhibit Non-monotonic Dose-Response to HFPO-DA**

***Jeanne P Vu*[1]**, *Flannery McLamb[2], Zuying Feng[2], Damian Shea[3], Richard M. Gersber1[1] and Goran Bozinovic[4], (1) San Diego State University, (2) Boz Life Science Research and Teaching Institute, (3 )Statera Environmental, Inc., (4) University of California, San Diego*

Per- and polyfluoroalkyl substances (PFAS) are persistent pollutants known for their bio-accumulative properties and prevalence in water supplies and household products. Although legacy PFAS, such as perfluorooctanoic acid, are phased out in the U.S. due to public health concerns, a PFAS variant hexafluoropropylene oxide-dimer acid (HFPO-DA) is an emerging replacement. HFPO-DA is a potential neurotoxicant that has been shown to cause dopaminergic neurodegeneration. We investigated the bioaccumulative potential of HFPO-DA and its effects on lifespan, locomotor activity, and brain gene expression in female and male *Drosophila melanogaster* (fruit flies). Flies were collected less than 4 hours after eclosion and diet-exposed to 0, 10, $10^2$, $10^3$, or $10^4$ mg/kg/day HFPO-DA. To measure the effect of HFPO-DA on lifespan, the numbers of surviving flies from each exposure were recorded every 24 hours. Flies were subjected to a negative geotaxis assay at 3, 7, and 14 days of exposure to measure the effects of acute, sub-chronic, and chronic exposures on locomotor ability. To capture HFPO-DA-induced sexually dimorphic gene expression responses in the brain we sequenced brain-specific mRNA from flies for 3, 7, or 14 days. The Bioconcentration Factor was 0.031 for females, and 0.026 for males. Dose and median lifespan were negatively correlated in both female ($R^2_{adj} = 0.77$; $p<0.0001$) and male flies ($R^2_{adj} = 0.77$; $p<0.0001$). Log-rank Mantel-Cox tests and one-way ANOVAs revealed that median lifespan was reduced in females starting at 10 mg/kg/day ($p<0.01$) and in males starting at $10^2$ mg/kg/day ($p<0.01$). Acute exposure at 10 mg/kg/day significantly decreased locomotor ability in females ($p<0.0001$) while acute exposures at 1 mg/kg/day decreased locomotor activity in males ($p <0.0001$). Among 7500 genes analyzed, pairwise gene expression comparison between controls and treatments identified 2496 differentially expressed genes using shallow RNA sequencing. Both locomotor ability and brain gene expression exhibited non-conventional dose-response, similar to patterns reported in endocrine disrupting chemical exposures. While HFPO-DA does not readily bioaccumulate in fruit fly bodies, high-dose exposures have sex-specific effects on lifespan, locomotor ability, and brain gene expression.

## 1.01A Adopting Alternative Approaches to Animal Testing for Ecotoxicity Assessments: Approaches and Avenues for the Future

### 1.01A.T-01 Control Performance of Amphibian Metamorphosis Assays with Xenopus laevis

***Raechel Puglisi***[1]*, James Wheeler*[2]*, Adriana Bejarano*[3]*, Jeffrey C. Wolf*[4]*, Scott Glaberman*[5]*, Laurent L.-M. Lagadic*[6]*, Zhenglei Gao*[6]*, Fiona Sewell*[7]*, Constance Mitchell*[1]*, Scott G. Lynn*[8] *and Michelle Rau Embry*[1]*, (1 )Health and Environmental Sciences Institute (HESI), (2) Corteva Agriscience, Netherlands, (3) Shell Health - Americas, (4) EPL, Inc., (5 )George Mason University, (6) Bayer AG - Crop Science Division, Germany, (7) NC3Rs, United Kingdom, (8) U.S. Environmental Protection Agency*

Evaluation of endocrine activity in human and wildlife involves specific assays used to evaluate relevant pathways. Multiple *in vivo* OECD and U.S Environmental Protection Agency's (EPA) test guidelines have been validated for mammals, amphibians, or fish, focusing on the estrogen, androgen, thyroid, and steroidogenesis (EATS) pathways. However, these assays often require the use of a substantial amount of laboratory animals, which are cost inefficient and contradict the 3R principles for animal welfare. Additionally, there are an increasing number of mandates worldwide moving towards an "animal free" testing paradigm.

One such assay, the amphibian metamorphosis assay (AMA), is an *in vivo* screening tool to assess potential interactions with the amphibian thyroid system. Larvae are exposed for 21-days, at day 7 and day 21, individuals are assessed for development and growth metrics. Thyroid gland histopathology is performed at test termination.

This presentation presents data from 52 test guideline studies: 46 performed in order to satisfy test orders from the U.S. EPA's Endocrine Disruptor Screening Program (EDSP) and 6 additional studies that were more recently performed. Data Evaluation Records for the EDSP studies and the data from the additional sponsored studies were collated to assess the typical control variability and performance of test. Reliable historical control data ranges will be developed for the core study endpoints and the associated validity and performance criteria. Additionally, the historical control data will be explored to investigate cross-laboratory and study differences, sources of variability, relationships between endpoints and test concentrations, and the power of the test design.

In summary, the overarching aim is to form a knowledge base that could be used to improve test performance and interpretation of data from the assay.

*This abstract does not necessarily represent US EPA policy.*

### 1.01A.T-02 Estimating Transcriptomic Points-of-departure (tPODs) in Embryonic Rainbow Trout Exposed to Benzo[a]pyrene

***Alper James Alcaraz***[1]*, Sydney Murray*[1]*, Phillip Ankley*[1]*, Bradley Park*[1]*, Katherine Raes*[1]*, Shakya Kurukulasuriya*[1]*, Anita Masse*[1]*, Doug Crump*[2]*, Nil Basu*[3]*, Markus Brinkmann*[1]*, Markus Hecker*[1] *and Natacha S. Hogan*[1]*, (1) University of Saskatchewan, Canada, (2) Environment and Climate Change Canada, (3) McGill University, Canada*

New approach methods (NAMs) are urgently needed to address the high cost, low throughput, and significant ethical concerns associated with the current toxicity testing frameworks. However, developing NAMs requires careful calibration through multiple lines of evidence. This study aimed to validate the use of transcriptomic points-of-departure (tPODs) from short-term rainbow trout (RBT; *Oncorhynchus mykiss*) embryo assay to estimate chronic apical POD. Embryos of RBT were exposed for 4 to 28 days post-hatch (dph) to graded concentrations of benzo[a]pyrene (B[a]P; measured: water control, 0.01% DMSO solvent control, 0.079, 0.35, 1.5, 7.4, and 28.6 μg/L), representing a model legacy PAH. Benchmark dose analysis of toxicogenomic data (RNASeq) at 4 dph yielded tPODs of 0.02, 0.15, 1.8, and 0.07 μg/L B[a]P for the median of the 20 most sensitive active genes, $10^{th}$ percentile of all active genes, mode of the first peak of gene-level benchmark doses, and pathway-level tPOD, respectively. After 28 days, morphometric analysis showed significant growth inhibition at >7.4 µg/L B[a]P, with notable decreasing trend in body weight. Molecular pathways, biochemical responses, histological alterations, and physiological responses supported the canonical B[a]P toxicity pathway model. This study showed that molecular perturbations at 4 dph lead to biological responses at more advanced life stages, providing a strong support in the use of tPODs at 4 dph to estimate chronic apical POD. This study is part of the EcoToxChip project (www.ecotoxchip.ca).

**1.01A.T-03 Innovative Approach to Acute Fish Toxicity Testing: Adaptation of Available In Vitro Methodologies**

***Tamara Dawn Lunsman**, Corteva Agriscience*

Traditionally, agrochemical toxicity is evaluated using numerous guideline toxicity tests, spanning many species including fish, invertebrates, birds, wild mammals, and other organisms. With potential agrochemical presence in surface waters due to runoff or drift, fish toxicity data are required to assess potential hazards to aquatic vertebrates. Many animals are used every year to characterize effects on aquatic vertebrates and *in vivo* testing is the current standard to assess off-target toxicity to fish. The most widely performed test is the acute fish toxicity test (AFT), according to OECD test guideline (TG) 203. With the availability of emerging technologies, ecotoxicity tests that use alternatives to conventional vertebrate organisms (i.e., *in vitro* testing) and new approach methodologies (NAMs) paves the way for reduced or animal-free approval procedures. Indeed, *in vitro* alternatives for fish testing supports fulfilling the 3R (Refinement, Reduction, and Replacement) principles and supporting animal welfare regulation (e.g., Directive 2010/63/EU). The RTgill-W1 (rainbow trout gill cell line) is a fish cell line derived from the epithelial gill cells of rainbow trout (*Onchorhynchus mykiss*) that was used in the development of the OECD TG 249 in 2021 and provided an *in vitro* alternative to *in vivo* testing to predict AFT. As an adaptation to the OECD TG 249, an innovative approach was explored that leveraged the RTgill-W1 cell line to measure luminescent ATP in an *in vitro* cytotoxicity test. This NAM provides an automated and high-throughput method and results were compared to OECD TG 249 results using a similar chemical validation set. The concordance between AFT results and the adapted OECD TG 249 suggests that this *in vitro* NAM represents a fast and efficient alternative to *in vivo* fish toxicity screening.

**1.01A.T-04 Does Age Really Matter? Examining Age-Specific Proteomic and Behavioral Responses of Zebrafish (Danio rerio) to a Model Toxicant**

***Abigail Henke**, Kevin Stroski, Laura M. Langan and Bryan W. Brooks, Baylor University*

Toxicological safety data for environmental contaminants is necessary to characterize hazards and risks to both human and ecological health, yet is deficient for most existing chemicals. These deficiencies, coupled with the significant time and monetary costs of traditional toxicity testing, have prompted a shift towards methods that reduce the number of animals, leverage existing data, and implement high throughput screening (HTS) techniques. Due to their high fecundity, rapid development, and sensitivity, current in vivo screening measures commonly utilize embryonic fish as toxicity models. Zebrafish (Danio rerio) share a high degree of genetic similarity with humans (>70% of human genes have a zebrafish ortholog), making them a popular model for human health and toxicological studies such as the fish embryo toxicity (FET) test. While the FET test typically exhibits a high level of correlation with the traditional adult Acute Fish Toxicity test (AFT), there is evidence that FET and AFT can demonstrate differing sensitivities to some compounds. Previous research from our lab identified that larval zebrafish exposed to the antihistamine diphenhydramine (DPH) demonstrate increased mortality, uptake, and behavioral toxicity when compared to the embryonic stage. However, whether molecular initiation events influenced such age-specific differences was not investigated.

We examined this research gap by analyzing changes in protein expression and behavior of zebrafish exposed to DPH at sublethal concentrations across embryonic and early larval stages. Analysis of photolocomotor behavior confirmed previously observed dose-dependent responses to DPH, and age-specific responses differed following DPH exposure. Proteomic analysis identified >3200 proteins (at 1% false discovery rate), which were grouped by gene ontology resulting in differential expression of proteins implicated in morphogenesis, angiogenesis, and neural development. When we performed age specific functional comparisons, genes associated with chemoattraction, neuron generation, cardiac development, and ion gated channel activity showed differences in expression across developmental age. By contrasting changes in behavior and whole-body protein expression across different developmental and exposure conditions, this study provides a basis to further examine how age-specific sensitivities affect early life-stage response to toxicants.

**1.01A.T-05 Evaluating Correlations of ToxCast and ECOTOX Data Through Toxicity Benchmark Derivation**

***Christopher M. Schaupp***[1]*, Erin Maloney*[2]*, Jennifer Olker*[3] *and Daniel L. Villeneuve*[3]*, (1) Oak Ridge Institute for Science and Education (ORISE) participant at U.S. Environmental Protection Agency, (2) University of Minnesota, (3) U.S. Environmental Protection Agency*

Large repositories of new approach methodology (NAM)-based data, such as the high-throughput, mammalian-centric *in vitro* bioactivity data in the EPA's Toxicity Forecaster (ToxCast), provide a wealth of publicly accessible toxicity information for thousands of chemicals. These data can be used to calculate point-of-departure (POD) estimates via concentration-response modeling that may serve as lower bound, protective estimates of *in vivo* effect. However, discussions about the utility of these data have largely focused on potential integration into human hazard assessment, and their application to ecological risk assessment remains limited. The goal of the present study was to compare and contrast PODs based on the 5$^{th}$ centile of the activity concentration at cutoff (ACC) in ToxCast with the distribution of *in vivo* PODs compiled in the Ecotoxicology Knowledgebase (ECOTOX). ECOTOX, "the world's largest compilation of curated ecotoxicity data," contains thousands of *in vivo* and *in vitro* toxicity datapoints relevant to ecological receptors. Preliminary results showed poor overall correlation between ToxCast and ECOTOX PODs for 636 chemicals. However, parsing by chemical classification revealed certain classes of compounds show good correlation (e.g., pharmaceuticals; $R$ = 0.49, $p$ = 0.0037), while others, such as organophosphate insecticides ($R$ = 0.047, $p$ = 0.76), do not. Surprisingly, these trends were mostly unaffected when the derivation of ECOTOX PODs was faceted by apical vs. biochemical endpoints, acute vs. chronic data, and study species. Future analyses will investigate ToxCast and ECOTOX benchmark correlation(s) by mode-of-action. Results of this research help to define potential utility and limitations of ToxCast data for predicting ecological hazards. *This abstract neither constitutes nor necessarily reflects USEPA policy.*

**1.01A.T-06 Developing a New Approach to Assess Crop Protection Chemical Safety That Minimizes Reliance on Vertebrate Testing While Protecting the Environment**

***David A. Dreier****, Richard Currie, Haitian Lu, Ramanarayanan Tharacad, Natalia Ryan, Odette Alexander, Douglas Wolf and John Abbott, Syngenta*

USEPA's directive to reduce animal testing has implications for ecological risk assessment, as several vertebrate tests are used to support these assessments, namely in fish and birds. Accordingly, Syngenta is developing an approach to generate the information necessary to address these key knowledge needs without the use of chemical-specific vertebrate tests. We began with a case study for a new ACCase inhibitor herbicide with read-across potential. To assess hazard across species, we followed a tiered approach and compared acute toxicity endpoints across several taxonomic groups, including fish, invertebrates, and plants. Specifically, we extracted data from USEPA ecological risk assessment documents and found that there were no significant differences across taxa for existing ACCase inhibitor herbicides, but there were differences in toxicity as a function of chemical class. Next, we constructed chemical toxicity distributions that quantify the probability an ACCase inhibitor would have acute or chronic effects in fish. An exposure distribution was also constructed using the USEPA Pesticide in Water Calculator to estimate surface water exposure for a broad selection of GAPs that cover various corn and soybean uses. Finally, these distributions of exposure and effect were integrated using a joint probability curve, which indicated de minimus risk of adverse effects in fish. This analysis, performed using only existing data and statistical methods, was able to demonstrate extremely low risk to fish by a new chemical, and we propose will negate the need to conduct any studies in fish species. While this example pertains to a new ACCase inhibitor, we emphasize this approach of making risk assessment predictions based on existing data can be extended to other active ingredients and chemical classes, as well as other taxonomic groups of interest.

**1.01A.T-07 Bioaccumulation Screening of Neutral Hydrophobic Organic Chemicals in Air-Breathing Organisms Using In Vitro Rat Liver S9 Biotransformation Assays**

***Yung-Shan Lee****, Talia Cole, Manpreet Jhutty, Mark Cantu, Beatrice Chee, Stephanie Stelmaschuk and Frank Gobas, Simon Fraser University, Canada*

To advance methods for bioaccumulation assessment of organic substances in air-breathing organisms using alternative approaches to animal testing, this study developed an in vitro approach for screening neutral hydrophobic organic substances for their bioaccumulation potential in air-breathing organisms consisting of (i) depletion assays for chemicals in rat liver S9 subcellular fractions; (ii) in vitro-in vivo extrapolation (IVIVE); and (iii) whole organism bioaccumulation modeling to assess the biomagnification potential of neutral organic substances in the rat. Testing of the in vitro method to 14 test chemicals of potentially biomagnifying substances showed that the bioassays could be conducted with a high level of reproducibility and that in vitro-derived elimination rate constants were in good agreement with in vivo-determined elimination rate constants in the rat. Exploring the potential of the in vitro approach for screening organic chemicals for bioaccumulation in air-breathing organisms indicated that chemical substances that exhibit a depletion rate constant in the S9 in vitro bioassay equal to or greater than 0.3 $h^{-1}$ are not expected to biomagnify in rats independent on their octanol-water partition coefficients ($K_{OW}$) or octanol-air partition coefficients ($K_{OA}$). The high level of reproducibility achieved in the test, combined with the good agreement between in vitro-derived and in vivo-determined depuration rate suggest that the in vitro approach in combination with a $K_{OA}$ and $K_{OW}$ based screening approach has good potential for screening chemicals in commerce for their bioaccumulation potential in air-breathing organisms in a cost effective and expedient manner, especially if the bioassay can be automated. The developed methodology can inform a weight of evidence approach to support regulatory bioaccumulation assessment as well as environmental hazard and risk assessments.

**1.01A.T-08 Development and Characterization of a Double-Crested Cormorant Hepatic Cell Line for Chemical Screening**

***Tasnia Sharin****[1], Doug Crump[2] and Jason O'Brien[2], (1) University of Ottawa, Canada, (2) Environment and Climate Change Canada*

Double-crested cormorants (DCCO) are high trophic level aquatic birds that are used for routine contaminant monitoring programs in the Laurentian Great Lakes and marine coasts of Canada. Primary embryonic hepatocyte culture is a commonly used *in vitro* model for avian toxicity testing. However, it is difficult to obtain eggs of avian wildlife species for the preparation of primary embryonic hepatocytes. Thus, it is a challenge to screen large numbers of chemicals using primary hepatocytes of wild species. There are currently no commercially available cell lines for DCCO. In this study, an immortalized DCCO hepatic cell line, DCH22, was established from the liver of a day 22 female embryo as a potential alternative to double-crested cormorant embryonic primary hepatocytes (DCEH) for chemical screening. For chemical exposure experiments, DCH 22 cells were cultured as 2D monolayer and 3D spheroids. CYP1A activity was measured using the ethoxyresorufin-*O*-deethylase (EROD) assay and CYP3A activity was determined using the P450-Glo CYP2C9 and CYP3A4 Assays and Screening System. DCH22 cells were cultured for over a year and have epithelial-like morphology. Exposure to four CYP1A inducers, benzo(a)pyrene, PCB-126, β-napthoflavone and phenacetin, for 24h led to an increase in EROD activity in both 2D monolayer cells and 3D spheroids. CYP3A activity increased in 2D monolayer cells and 3D spheroids upon exposure to increasing concentrations of four CYP3A inducers, hexabromocyclododecane, tris(1,3-dichloroisopropyl)phosphate, carbamazepine and metyrapone. In general, CYP1A and CYP3A induction was lower in 2D monolayer cells compared to 3D spheroids. Induction of CYP1A and CYP3A activities in both culture conditions indicate that the DCH22 cell line has metabolic competence. Species-specific RT-qPCR will be used to measure changes in mRNA expression of genes involved in xenobiotic metabolism following exposure to CYP1A and CYP3A inducers. The cells will be further characterized by measuring albumin and vitellogenin levels using ELISA assays. Preliminary results suggest that this cell line may be a suitable alternative to primary hepatocytes for avian chemical screening.

**1.01B Adopting Alternative Approaches to Animal Testing for Ecotoxicity Assessments: Approaches and Avenues for the Future**

**1.01B.T-01 Evaluating the RTgill-W1 Cytotoxicity Assay Against Polymers**
***Kyle Roush*** *and Kristin A. Connors, Procter & Gamble*
Regulatory data exemptions regarding polymers are currently being re-evaluated, and generation of fish toxicity data is expected as part of chemical safety assessments. Given concerns around animal testing, alternative methods for acute fish toxicity (AFT) will be critical to fulfilling substantial data gaps in environmental hazard and risk assessment. Publicly available data on alternative methods tested against polymers is limited, with literature focusing on the fish embryo toxicity test (FET). FET tests of non-ionic and cationic polymers have suggested that alternative assays are sensitive to polymers and may display unique physical effects as a result of exposure. However, other alternatives need to be investigated to build the weight-of-evidence for AFT data replacement. Fish gill cells have been proposed as a key in vivo target tissue of aquatic chemical exposures, thus leading to development of a standardized rainbow trout gill cell line (i.e., RTgill-W1) toxicity assay. Though validated as predictive of AFT against a diverse set of chemicals, polymer toxicity assessment via this assay has yet to be explored. As such, the goal of this study was to assess the sensitivity and utility of the standardized RTgill-W1 cell toxicity assay in predicting acute fish toxicity resulting from polymer exposure. Tests of non-ionic polyethylene glycols (PEGs) and cationic polyquaterniums (PQs) were conducted according to ISO 2115/OECD 249. Materials were selected to encompass a range of backbones, molecular weight, and charge density, and confirmed to have available FET and AFT data for comparison. Toxicity was assessed after 24-hour exposures utilizing fluorescence of selective dyes indicative of cell viability. Results indicate that the assay may not be as sensitive (i.e., toxicity detected at higher concentrations) as AFT tests or other alternative assays (i.e., FET tests) without adaptations to the standard test protocol. Findings from this study help determine the broader utility of the gill cytotoxicity assay by defining relevant chemical applicability domains and identifying potential limitations of the standardized method. This is critical to supporting the comprehensive use of this assay and growing the portfolio of alternative methods for generating acute fish toxicity data.

**1.01B.T-02 Can Tests with Fish Embryos or Shrimp Replace Larval Fish Tests? - An Initial Evaluation of Marine Alternatives**
***Dalton Scott Allen****, Michaela Kelly, Maddie Wiencek and Marlo K Sellin Jeffries, Texas Christian University,*
Current regulations require that effluents discharged into aquatic receiving environments be assessed for potential toxicity often using standardized fish-based testing methods. Though these methods have proven effective in identifying toxic effluents and mitigating their associated adverse effects, recent legislation demands that alternatives promoting animal welfare be utilized whenever possible. This has spurred the development of alternative testing strategies, such as the zebrafish embryo toxicity (FET) test, for use in freshwater toxicity assessments. However, there are a lack of analogous alternatives for marine effluent testing. As such, the goal of this study was to improve the welfare of fish utilized in marine effluent toxicity tests by comparing two commonly utilized standardized test methods (the sheepshead minnow and inland silverside larval growth and survival (LGS) tests) to three potential alternative test methods (the sheepshead minnow and inland silverside FET tests and the mysid survival and growth test). To accomplish this, each toxicity test was utilized to evaluate the toxicity of 3,4-dichloroaniline (DCA), a common reference toxicant. The resultant toxicity values, including median lethal concentrations (LC50s), were determined and compared between the test types. The results of this comparison showed no significant differences between the LC50 values

determined via the inland silverside LGS, sheepshead minnow LGS, and mysid tests. However, the LC50 values derived from the inland silverside and sheepshead minnow FET tests were found to be significantly higher than the other test types indicating reduced sensitivity relative to the other test types. Based upon the results of these comparisons alone, the mysid test appears to represent a comparable alternative to the LGS tests for acute toxicity testing. Though the FET tests were less sensitive than the LGS tests, this does not necessarily preclude the FET test as a viable alternative as a predictive relationship between the test types may exist; though, future research using a broader spectrum of test chemicals would be needed to establish such a relationship.

**1.01B.T-03 Evaluating Metabolomics as a New Approach Method (NAM) for Toxicity Testing and Environmental Monitoring: An Assessment of IVIVE in Zebrafish**

***Drew R. Ekman****, Jonathan D. Mosley, Timothy W. Collette, Jenna E. Cavallin, Daniel L. Villeneuve and Quincy Teng, U.S. Environmental Protection Agency*

The development of new approach methodologies (NAMs) to assess human and ecological toxicity to priority chemicals is an active area of research for national and international regulatory agencies. Recently, efforts have begun to utilize NAMs for evaluating biological impacts from complex contaminant mixtures to support risk assessments by EPA's Regional and Program Offices. Due to the complexity of these exposures, it has been recognized that successful implementation will require high throughput approaches capable of collecting large numbers of relevant endpoints in an untargeted fashion. Metabolomics has been identified as a promising approach and is now a proven untargeted technique for capturing *in vitro* responses to a wide variety of contaminant mixtures in surface waters. However, there remains a dearth of information regarding the strengths and limitations of common *in vitro* models for translating metabolomic responses to *in vivo* systems (i.e., *in vitro* to *in vivo* extrapolation or IVIVE). Zebrafish are increasingly being used as a model test species for evaluating the toxicity of priority contaminants and for assessing risks associated with exposures to complex mixtures in the environment. This includes the use of the zebrafish model with both *in vivo*- and *in vitro*-based test systems. Here we present an untargeted mass spectrometry-based comparison of the hepatic metabolomes collected from unexposed (i.e., control) lab-reared adult zebrafish and the zebrafish liver cell line (ZFL) cultured in clean (i.e., control) media. This analysis revealed metabolites and biochemical pathways that are shared between the *in vitro*- and *in vivo*-based test systems, and thus more useful for extrapolation and prediction of adverse outcomes in exposure scenarios. Conversely, metabolites and biochemical pathways that are not shared (specifically those only found *in vitro*) are also identified and recommended for deprioritization for effects-based testing. *The contents of this abstract neither constitute nor reflect US EPA policy.*

**1.01B.T-04 Sex-dimorphism in Hepatic Transcriptomic Responses of Embryonic Japanese Quail to Ethinylestradiol**

***Yeon Seon Jeon**[1], Ejimedo Madogwe[1], Emily Boulanger[1], Raj Duggavathi[1], Doug Crump[2] and Jessica Head[1], (1) McGill University, Canada, (2) Environment and Climate Change Canada*

Early-life stage toxicity tests are considered alternatives to those performed in adults. Our recently proposed early-life stage test for birds uses embryos of mixed sex in order to avoid doubling the sample size. Sex, however, is often considered a critical factor to control for toxicological studies, especially when endocrine disruption is the focus. To assess the effect of sex in the avian early-life stage toxicity test, we explored sex-dimorphic gene expression of embryonic Japanese quail after ethinylestradiol (EE2) exposure. Exposures were conducted according to an avian egg injection protocol that we have proposed for standardization. EE2 was dissolved in dimethyl sulfoxide and injected into the air cell of eggs prior to incubation at nominal concentrations of 0 and 3.33 µg/g egg weight. At mid-incubation (embryonic day 9), liver tissues were collected from 5 embryos per sex per treatment group for RNA-sequencing. Differential expression analysis was performed on males and females separately or grouped together. Principal component analysis revealed clear sex-dimorphism, but the differentiation between control and treatment groups was also apparent. Following exposure to EE2, male and female embryos had 272 and 681 differentially expressed genes (DEGs; log2 fold

change > 1, adjusted $p$-value < 0.05), respectively, with 143 DEGs in common between males and females. The shared DEGs included known biomarkers for estrogenic exposure, such as vitellogenin genes and apovitellin-1. Overall, our results suggest that transcriptomic signatures of estrogen exposure are apparent in mixed-sex experiments, despite sex-dimorphic responses. This study informs the standardized early-life stage test for birds and contributes to the development of screening methods for estrogenic chemicals using avian embryos and transcriptomic approaches.

**1.01B.T-05 Tiered Methods for Bioaccumulation Assessment to Reduce Animal Testing**
***Liisa Toose***[1]*, Alessandro Sangion*[2]*, James M. Armitage*[3]*, Michelle Rau Embry*[4] *and Jon A. Arnot*[1]*, (1) ARC Arnot Research & Consulting Inc., Canada, (2) University of Toronto Scarborough, Canada, (3) AES Armitage Environmental Sciences, Inc, Canada, (4) Health and Environmental Sciences Institute (HESI)*
Bioaccumulation (B) assessments using various methods, data, metrics, and classification criteria are routinely conducted as a part of chemical regulatory programs. B assessments are challenging because of limitations on, and availability of reliable and representative empirical data. Here, we present tiered methods for B assessment to reduce unnecessary animal testing. The Bioaccumulation Estimation Tool (BET) is a screening-level modelling system integrated in the Exposure And Safety Estimation (EAS-E) Suite platform. The BET includes mass balance bioaccumulation models for a diverse range of representative ecological receptors (plants, invertebrates, fish, birds, and mammals) and food webs (aquatic and terrestrial). Typical laboratory test animals are also included, i.e., fish and rat. The BET is automatically parameterized in the EAS-E Suite platform requiring only chemical name, CAS or SMILES notation as input. Users can easily refine the default input parameters with preferred partition coefficients (including options for biological-phase partitioning), biotransformation rates (including in vitro rates from S9, hepatocyte or microsomal assays) and dietary absorption efficiencies. The BET calculates B metrics such as lab fish and invertebrate Bioconcentration Factors (BCFs), field Bioaccumulation Factors (BAFs) for invertebrates and fish as well as lab and field fish and homeotherm Biomagnification Factors (BMFs) based on uptake, accumulation, and elimination kinetics (i.e., total elimination half-life). The same screening-level models in the BET are included in the Bioaccumulation Assessment Tool (BAT). The BAT guides the collection, generation, evaluation, and integration of various Lines of Evidence (LOE) (in silico, in vitro, lab and field studies) for a higher-tiered weight-of-evidence approach for B assessment. A case study showing the applicability of the BET and the BAT for the B assessment is presented. The BET Ver.0.95 and the BAT Ver.2.02 are freely available and can readily be used by interested stakeholders from academia, industry, and the regulatory community.

**1.01B.T-06 Exploring Ways to Implement the 3Rs (Reduction, Replacement, Refinement) Principles in a Regulatory Context**
***Michael Lowit****, U.S. Environmental Protection Agency*
The U.S. Environmental Protection Agency (USEPA) is applying the 3Rs (reduction, replacement, refinement) to the ecological risk assessment process in working toward the goal of reducing animal testing while ensuring protection of the environment, which is the mission of the USEPA. The USEPA Office of Pesticide Programs (OPP) standard approach for the registration of conventional pesticides is to base ecological risk assessments on toxicity data primarily derived from whole animal studies, as required in the code of federal regulations (40 CFR part 158). However, OPP has a longstanding commitment to the 3Rs principles and has the flexibility to grant study waivers and incorporate alternative approaches into the ecological risk assessment process. To advance these efforts, OPP is conducting systematic retrospective analyses of the *in vivo* ecotoxicity studies required by the USEPA for registration. The purpose is to critically evaluate which guideline studies form the basis of regulatory decisions and whether the number of animals used can be reduced without a reduction in the confidence in the assessment of ecological risk. OPP is also exploring the role of *in silico* approaches in making decisions about data needs for fish and mammals and is looking ahead to the possibility of integrating other new approach methods (NAMs) that are fit for purpose in addressing regulatory needs. This presentation will highlight the results of recently completed, active, and future retrospective analyses on acute and chronic

toxicity to fish and birds and will discuss the potential use of alternative approaches. The results of these efforts will inform whether there is a basis for reducing the number of *in vivo* animal tests conducted to support pesticide registrations.

**1.01B.T-07 Using the zebrafish embryo assay to evaluate the developmental toxicity of PFAS used in the photolithography industry**

***Yuexin Cao***, *Hajar Smaili, Ruiwen Chen and Carla A. Ng, University of Pittsburgh*

Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals with useful surface-active properties and resistance to harsh environments. They have been used for decades in a variety of industries, including photolithography. However, studies have shown that PFAS, especially long-chain PFAS, harbor bioaccumulative potential and may cause toxic effects on aquatic and terrestrial organisms. These adverse effects include immunotoxicity, reproductive and developmental toxicity, as well as various cancers. Increasing concerns regarding PFAS safety led to growing demand for toxicity data. However, traditional mammalian toxicity testing is time- and resource-consuming. The zebrafish embryo assay is developing as a popular alternative approach to more long-term and adult vertebrate tests. They are widely used as models for evaluating adverse effects of xenobiotics, and as proxies for acute fish toxicity testing because of the potential for higher throughput and relevance to both wildlife and human toxicity testing. In this study, zebrafish (*Danio rerio*) embryos were placed in 96-well plates and exposed to freshly spiked PFAS solutions for 120 hours to explore the developmental toxicity of PFAS used in photolithography. After exposure, larvae were assessed for survival, hatching, and developmental malformations, including curved body axis, failed swim bladder inflation, yolk sac edema and necrotic yolk sac. Our study generates new toxicological data on diverse PFAS in the form of dose-response relationships and/or no observed effect concentrations, together with correlations between observed effects and structural features. These data will inform the ongoing development of quantitative structure-activity relationships (QSARs) that incorporate molecular modeling and machine learning to predict the relative hazards of PFAS used in photolithography. Based on the ability to quickly generate data for multiple compounds, the zebrafish embryo assay is a powerful tool that serves as both a direct screen for toxicity and a means to provide structure-activity relationship data to aid in parameterization and validation of models.

**1.01B.T-08 Using the RTgill-W1 Cell Line to Investigate Cytotoxic and Molecular Effects of Sediment Extracts from the Agbogbloshie Electronic-Waste Site.**

***Krittika Mittal***[1], *Jingyun Zheng*[1], *Stéphane Bayen*[1], *Julius Fobil*[2] *and Nil Basu*[1], *(1) McGill University, Canada, (2) University of Ghana*

Electronic-waste (E-waste) sites are notoriously contaminated with complex chemical mixtures thus challenging environmental monitoring, management, and remediation activities. Additionally, there is increasing awareness that traditional whole animal based toxicity tests are resource-intensive, expensive and unethical. Given that E-waste sites are typically situated in low- and middle-income countries that tend to be poorly resourced, there is a need to develop more efficient techniques for application in such settings. The objectives of the current study were to A) perform simultaneous targeted analysis and non-targeted screening of plastic related contaminants in extracts prepared from 35 soil samples collected at the Agbogbloshie E-waste site (Accra, Ghana; classified as upstream-6, downstream-2, community-3, trade site-8, dump site-13, and burn site-3) through liquid chromatography coupled to hybrid quadrupole time of flight mass spectrometry (HPLC-QTOF-MS) in full scan mode, and B) to characterize the cytotoxic and molecular effects of these extracts on the rainbow trout (RT) gill cell line RTgill-W1, following the OECD test guideline #249. High concentrations of bisphenol A were measured ranging from 96.80 ng/g (in upstream, downstream, and community) to 255.62 ng/g dry weight (in trade site, dump site, and burn site). Other plastic-related chemicals such as phthalates were also detected, and the concentration of dibutyl phthalate was up to 384.83 ng/g dry weight (in trade site, dump site, and burn site). Gill cells were exposed to concentrations equivalent to 9.38, 4.69, 2.34, 1.17, 0.59, and 0.29 mg dry weight of extract (eQsed)/ml. Many of the samples from the various site types caused a decrease in cell viability % at 9.38

eQsed/ml, for example., two upstream and two community samples (6.9 - 66%), five dump site samples (5.2 – 31.4%), the eight trade site samples (2.2 – 53%), and two burn site samples (59.6 – 77.4%). The trade site group was the most cytotoxic, and the most cytotoxic sample was trade site #8 with cell viability dropping from 74.2% to 2.2% at 0.29 to 9.38 eQsed/ml. Further chemical analysis, and exposures to examine molecular effects of the sediment extracts on the gill cells are underway. This work is expected to support ongoing efforts in establishing the use of efficient alternative testing strategies in ecotoxicology with a focus on developing methods for use in contaminated sites in under-resourced locations.

## 1.02 Advancements in Aquatic and Wildlife Immunotoxicology: Innovative Approaches to Identifying Adverse Outcomes

### 1.02.T-01 Detection and Tracking of Inflammatory Constituents in Environmental Water Samples Using Immune Cell Lines

***Nora Hussain***[1]*, Dustin ME Lillico, Yemaya Choo-Yin, Rui Qin, Zuo Tong How, Sunanda Paul, Mohamed Gamal El-Din and James L. Stafford, (1) University of Alberta, Canada*

Large volumes of water are generated in sediment-bound bitumen extraction in oil sands mined sites. While these waters have various fates in the extraction processes (such as maintained in constructed reservoirs and recycled for subsequent extractions), ultimately, the constituents within oil sands process-affected water (OSPW) will need to be addressed to enable treated mine water release. These components may be organic or inorganic, with the former containing the naphthenic acid fraction compounds (NAFC), which are mainly considered responsible for toxicity. However, inorganic constituents such as ions and salt may also contribute to toxicity.

We have recently developed a cell-based assay to screen waters for inflammatory components by monitoring select immune markers indicative of immune cell activation. Using macrophage cell lines, we have optimized several bioactivity assays to detect and track constituents of potential concern within complex water samples. Macrophages represent a dynamic population of immune cells found in all vertebrates that sense foreign substances entering the body. These cells help capture and kill pathogens, and react to anthropogenic substances in aquatic and terrestrial environments. Macrophages reside in tissues exposed to the external environment (i.e., skin, gills, lungs, and the digestive tract), positioning them as sentinels for incoming environmental toxicants. Thus, macrophages represent an excellent model as innate immune cell-based biosensors to evaluate the potential effects of contaminated water exposures on fish and mammals.

Our macrophage bioassays have screened for inflammatory components in several waters such as OSPW, municipal tap water, and environmental freshwater. These experiments demonstrate that raw OSPWs induce a potent inflammatory response compared to tap water, freshwater, and OSPW treated using various remediation techniques including advanced oxidation processes (AOPs). AOPs degrade organic components such as the NAFC, suggesting that the inflammatory activity of raw OSPW is partly due to AOP-sensitive compounds like naphthenic acids. In addition, temporal sampling of treated OSPW in a demonstration pit lake, showed increased bioactivity mainly associated with the organic fraction. Overall, our work demonstrates a promising bioassay screening tool with the potential to be economically integrated into current toxicological frameworks following downstream standardization procedures.

### 1.02.T-02 Using Microfluidic Chip Technology to Enhance Immunotoxicity Testing

***Maria L Rodgers***[1]*, Abdelmounaim Nouri*[2]*, Chia-Ming Wang*[3]*, Natalie Steinel*[2]*, Kathryn Miligan-McClellan*[1]*, Rebecca Carrier*[3] *and Daniel Bolnick*[1]*, (1) University of Connecticut, (2) University of Massachusetts, Lowell, (3) Northeastern University*

A substantial challenge in aquatic immunotoxicology research is differential responses to immunotoxicants across taxa, making it difficult to extrapolate data for species other than the one(s) tested. However, testing every species for every possible immunotoxicant is an impossible task. The advent of organ-on-chip technology may provide a solution. Organs-on-chips are a high throughput technology that uses cells grown in specific conditions to mimic whole organs. We have developed an intestine-on-chip system for threespine stickleback fish (*Gasterosteus aculeatus*) that can be used to test toxicants of interest and understand their impacts on mucosal immunity in the intestine. The intestine is a particularly relevant organ to study in immunotoxicology because of its role in both immunity and in the absorption and metabolism of xenobiotics.

Our intestine-on-chip consists of a molded silicone polymer, polydimethylsiloxane (PDMS), attached to a glass coverslip. A hydrogel containing extracellular matrix protein (collagen) fills the middle channel of the chip (to promote the growth of epithelial cells), and two side channels of the chip act to flow nutrients into the closed system, while also flowing out waste. With this system, we can examine cell location, use RNA in situ hybridization (RNA-ISH) to visualize RNA, detect changes in the microbiome, and use the outflow of media/nutrients to determine the expression of important pro-and anti-inflammatory cytokines. Thus far, we have successfully seeded epithelial cells into the intestine-on-chip system and are currently working on adding in immune cells and microbiota (we have access to a library of over 300 microbes taken from various stickleback populations). In addition, we have cataloged the universe of intestinal cells in stickleback using single-cell RNA sequencing (scRNAseq). From scRNAseq we have also linked the expression of specific genes to certain cell types (e.g. *mpeg1.1* and macrophages), and have created eight different probes for use in RNA-ISH applications. Though we have focused on threespine stickleback so far, the design of the chip is well-suited for nearly any fish species, and we have even successfully used this chip with human and murine cells. This intestine-on-chip will allow us to screen potential immunotoxicants to identify signs of inflammation and inflammatory processes across a variety of aquatic taxa.

**1.02.T-03 Effects of Glyphosate and Perfluorooctanesulfonic Acid (PFOS) on the Immune System of the Florida Manatee (Trichechus manatus latirostris)**

***Maite De Maria***[1]*, Nicole Stacy*[1]*, Kevin J Kroll*[1]*, Francisco Barboza*[2]*, Cecilia Silva-Sanchez*[1,3]*, Catherine Walsh*[4]*, John A. Bowden*[1]*, Natalia Reyero*[5] *and Nancy Denslow*[1]*, (1) University of Florida, (2) University of Tartu, Estonia, (3) NCASI, Inc., (4) Mote Marine Laboratory, (5) U.S. Army Engineer Research and Development Center*

The immune system's role is to protect against infectious disease and antigens, although exposure to chemicals can suppress immune responses decreasing its ability to function normally. Glyphosate is the most used herbicide worldwide and it has been found in Florida waterbodies chronically exposing the Florida manatee. More than 50% of the manatees had glyphosate in their plasma. Florida manatees are also exposed to flame retardants such as Perfluorooctanesulfonic acid (PFOS) that has been quantified in their plasma. We performed RNA-sequencing from buffy coats from wild manatees exposed *in-vivo* simultaneously to glyphosate and PFOS (n=5) in comparison to individuals that had low concentrations of either contaminant (n=15) or had neither. Manatees exposed to both contaminants showed significant upregulation of genes related to stress and inflammation such as heat shock 70, heat shock 90, and TNF induced alpha protein 6 (TNFAIP6) and pathways significantly enriched in response to chemical substances, tissue repair and kidney tubule function. In *in-vitro* experiments, we isolated lymphocytes from blood samples of 12 free-ranging animals sampled in December-January 2018 and December 2019. Lymphocytes were exposed to 0, 10, 1000, and 10,000 µg/L of glyphosate and simultaneously to a mitogen (phytohemagglutinin 5 µg/mL). Glyphosate caused a dose-dependent reduction in the proliferation capacity of T-lymphocytes with a significant decrease at 10,000 µg/L of glyphosate with a mean reduction of 27%. The effect of glyphosate on the Florida manatee's immune system had a more pronounced effect in some individuals with a reduction of the proliferation capacity in response to a mitogen by 44.3% at 1,000 µg/L and 50.1 % at 10,000 µg/L of glyphosate. This data suggests that exposure of manatees to glyphosate may target their adaptive immune response by altering the ability of T-lymphocytes to proliferate. Glyphosate exposure in the environment may show synergistic effects with other contaminants such as PFOS.

Our findings should be considered in the context of a threatened species that faces other stressors affecting their immune response such as red tide and cold stress.

**1.02.T-04 Development of a New Infection Model for Fathead Minnows, an Emerging Immunotoxicity Model Organism**

*Kahler Doyle, Kyle Horton and **Marlo K. Sellin Jeffries**, Biology, Texas Christian University*

Emerging evidence suggests that the fish immune system is vulnerable to disruption in response to a wide variety of chemical contaminants; thus, there is a need to develop routine assays to test chemicals for immunotoxicity. In recent years, the fathead minnow (*Pimephales promelas*), a common model for the assessment of acute, chronic, and reproductive toxicity, has emerged as a model for immunotoxicity. To date, immunotoxicity studies using the fathead minnow as a model have paired them with the bacterial pathogen *Yersinia ruckeri* to investigate pathogen resistance and pathogen-stimulated immune responses following chemical exposure. While the use of *Y. ruckeri* has been effective in this context, its use is not favorable given that minnows must be intraperitoneally-injected with *Y. ruckeri* to develop an infection. Thus, the goal of this project was to develop a new host-pathogen system for fathead minnows by identifying a pathogen that induces infection via immersion. To do this, the ability of three species of bacteria – *Aeromonas allosaccharophila, Aeromonas sobria,* and *Flavobacterium columnare* - to infect adult fathead minnows via immersion was evaluated. These bacterial species were selected based upon their status as biosafety level 1 pathogens, environmental relevance, and previously-documented ability to infect other cyprinid species. Of the three bacteria evaluated, only *F. columnare* was shown to induce disease and mortality in fathead minnows and only if fish were fin clipped prior to immersion. In addition, results showed a stark difference between the infection and mortality rates between male and female fathead minnows. Overall, these results suggest that *F. columare* may be a suitable pathogen to utilize in immunotoxicity studies featuring fathead minnows and also demonstrate sex-specific differences in baseline immune function in this species. The results of this study can be utilized to further develop the fathead minnow as a standard model for immunotoxicity testing.

**1.02.T-05 Comparing the Respiratory Burst In Vivo, In Vitro, and Ex Vivo After Exposure to Per- and Polyfluoroalkyl Substances**

***Drake Phelps**, Haleigh Conley, M. Katie Sheats and Jeffrey A. Yoder, North Carolina State University*

The United States Environmental Protection Agency currently estimates that there are more than 12,000 per- and polyfluoroalkyl substances (PFASs), which are used to produce non-stick cookware, food contact materials, hydro- and oleophobic textiles, and more. Due to their unique chemistry, they are ubiquitous and persistent in the environment, making exposure to PFASs commonplace. It is estimated that 98% of Americans have detectable serum levels of multiple PFASs. These compounds have also been detected in wildlife, illustrating their wide-reaching impact. It is well established that these compounds are immunotoxic; however, previous research has focused largely on the effects of PFASs on the adaptive immune system, leaving a knowledge gap on what is known about the effects of these compounds on the innate immune system. To bridge this gap, we utilized an *in vivo* larval zebrafish model, an *in vitro* human neutrophil-like cell culture model, and primary neutrophils exposed to PFASs *ex vivo* to investigate innate immune function after exposure to environmentally relevant PFASs. The respiratory burst was measured as a functional readout of innate immune function. Neutrophils induce microbicidal reactive oxygen species through the respiratory burst to defend the host against pathogens. Data show that some PFASs are capable of inhibiting the respiratory burst *in vivo, in vitro*, and *ex vivo*. Potency was similar among the model systems, indicating potential evolutionary conservation. Current studies are exploring whether exposure to PFASs confers susceptibility to infectious disease, and what mechanisms may be responsible for this immunosuppressive phenotype.

**1.02.T-06 Effects of the Legacy Contaminant ,1-dichloro-2,2-bis(p-chlorophenyl) ethylene (p,p-DDE) on Host Molecular Pathways Stimulated by Viruses in Fathead Minnows**

***Tara Sabo-Attwood**[1], Muhammad Sohail[2], Sarah Robinson[1], Haley Johnson[3], Christopher Martyniuk[1] and Syed*

*A.M.A.S Eqani[4], (1) University of Florida, (2) University of Central Punjab, Pakistan, (3) University of Wisconsin, (4) Comsat University Islamabad, Pakistan*
Chemical exposures to aquatic life can increase disease susceptibility via immune disturbance which may lead to significant population declines. Several reports show that the primary metabolite of DDT, 1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene (p,p-DDE), can adversely impact the immune system of mammals but fewer studies have focused on these effects in aquatic vertebrates. Therefore, the objective of this study was to examine the effects of p,p-DDE on the immune system of a model aquatic organism, the fathead minnow (*Pimephales promelas*). For this study, fish were fed sinking pellets that contained oil (control) or p,p-DDE (333 ng/g food) for 7 days followed by injection with the viral analog Poly I:C (2 ug/g fish). After 48 hours, fish were euthanized and kidney tissues were collected and processed for histopathology and transcriptomic analysis. A subset of whole fish were collected for quantitation of p,p-DDE. Data from the body burden analysis showed that fish given DDE-containing food had 11.3 ng/g in whole body extracts. Histopathology analysis showed that most of the findings were common between all fish however the number of scattered lymphocytes within the renal proximal tubular epithelium and necrosis of the distal/collecting tubular epithelium seemed to be higher in fish exposed to p,p-DDE and Poly I:C. Data from the transcriptomic analysis revealed the number of genes with altered expression in kidney tissues were 106 and 148 for the p,p-DDE and Poly I:C only exposed fish, respectively, whereas 102 genes were significantly altered in the combined p,p-DDE + Poly I:C group. A total of 61 genes with altered expression were common to all treatment groups. Pathway enrichment analysis showed gene networks specific to kidney cell processes and function were upregulated by exposure to DDE whereas an overall decrease in the expression of gene transcripts related to the immune system was noted. Importantly, many of the immune genes identified were specific to immunological surveillance processes and were induced by Poly I:C alone, as expected. The observation that p,p-DDE caused repression of typical Poly I:C induced responses suggest an immunosuppression mechanism is at play. These data suggest that p,p-DDE can impair the typical host response to viruses, potentially leading to increased susceptibility to infections. Furthermore, the transcriptional profiles provide foundational data for the development of immune-focused adverse outcome pathways.

**1.02.T-07 Using Genomic Applications to Understand Wild Smallmouth Bass Immune Function in Response to Exposure to Environmental Stressors**

***Cheyenne Smith**[1], Christopher Ottinger[2], Heather L. Walsh[2], Vicki Blazer[2] and Patricia Mazik[2], (1) West Virginia University, (2) U.S. Geological Survey*
A multidisciplinary approach has been implemented over the past two decades to investigate fish health issues in the Chesapeake Bay watershed. This effort has focused on smallmouth bass *Micropterus dolomieu*, which is a popular sport fish and has been disproportionately affected due to their sensitivity to pollution and other stressors. Considering there are multiple stressors affecting wild fish populations, the overall approach integrates chemical exposure, climatic factors, and biological indicators at long-term monitoring sites with differing degrees of land-use and chemical inputs to assess potential adverse exposure effects and cumulative risk. Immune function via *in vitro* immune function assays is being assessed as one of the biological indicators to determine if immunosuppression relating to a complex mixture of stressors is playing a role. The assays were integrated into comprehensive fish health assessments to monitor and evaluate changes in immune status in context with other aspects of health. During the assessments, anterior kidney tissues were collected for immune function and at the same time blood was taken for plasma analysis and multiple tissues were collected for histopathological indicators like parasite and pathogen prevalence and also preserved for gene expression. We are beginning to incorporate genomic applications to help understand mechanisms behind immune function results by looking at transcript abundance of immune- and contaminant-related genes from archived anterior kidney and liver tissues from previous field samples. Immune function results alone have revealed yearly, seasonal, site and individual differences but we need to consider these results in the context of the whole organism to interpret the significance. We have observed correlations between immune function and microscopic indicators such as parasite prevalence and macrophage aggregates in liver and spleen tissues plus

plasma concentrations of per- and polyfluoroalkyl substances but have not yet looked at correlations with gene expression. Correlations between immune function, various stressors, and transcript abundance of immune-related genes will be used to integrate lower-level responses like gene expression with higher-level responses like immune function to help us identify and ultimately understand adverse outcomes.

**1.02.T-08 Discussion: Immunotoxicology**
***Marlo K. Sellin Jeffries****, Texas Christian University*
This session will conclude with a discussion intended to share exciting developments aimed at supporting SETAC members with interests in immunotoxicology and kick start conversations regarding the current status and future of the field. In particular, the formation of the new SETAC Immunotox Interest Group (IGs) will be announced and upcoming IG activities will be discussed. Session participants will be encouraged to share how the new IG can best serve their interests and advance environmental immunotoxicology as a discipline. As part of this discussion, current obstacles in the field will be identified and ideas for how the Immunotox IG can be best used to address such obstacles will be explored. Though this discussion, we hope to welcome SETAC members to the new Immunotox IG, continue to build a community of environmental immunotoxicologists within SETAC, and collectively identify ways to advance aquatic and wildlife immunotoxicology research.

**1.02.P Advancements in Aquatic and Wildlife Immunotoxicology: Innovative Approaches to Identifying Adverse Outcomes**

**1.02.P-Mo019 How Do Per- And Polyfluoroalkyl Substances (PFAS) Affect Macrophage Function?**
***Ashley Connors*** *and Jeffrey A. Yoder, North Carolina State University*
Immune function can be impaired by environmental contaminants. One class of chemicals recently shown to interfere with the innate immune system is per- and polyfluoroalkyl substances (PFAS). For example, we reported that certain PFASs can reduce the oxidative burst *in vivo* in larval zebrafish (*Danio rerio*), *in vitro* in a human neutrophil-like cell line, and *ex vivo* in primary human neutrophils. To complement these neutrophil studies, we are evaluating how macrophages are affected by a 2-day (*in vitro*) and 4-day (*in vivo*) exposure to ten different PFASs: Perfluorobutanesulfonic acid (PFBS), Perfluorohexanesulfonic acid (PFHxS), Perfluorooctanesulfonic acid (PFOS), Perfluorohexanoic acid (PFHxA), Perfluorooctanoic acid (PFOA), Perfluorononanoic acid (PFNA), Perfluorodecanoic acid (PFDA), Nafion Byproduct 2, Perfluoro-2-methoxyacetic acid (PFMOAA), and Hexafluoropropylene oxide dimer acid (HFPO-DA or GenX). In cytotoxicity studies with macrophage-like THP-1 cells, we found no changes in cell viability at or below exposures to 80 µM PFAS. However, PFDA, PFNA, PFOS, and Nafion Byproduct 2 all led to drops in viability at 320 µM. We are currently investigating how phagocytosis is affected during PFAS exposures using both zebrafish larvae and THP-1 cells: macrophage populations derived from zebrafish and THP-1 cells will be challenged with fluorescent heat-killed *E. coli*. Phagocytic index and number will be measured with flow cytometry. Based on these functional assays, we will select 2-3 specific PFASs for studies that will elucidate currently unknown molecular mechanisms of PFAS immunotoxicity. To this end, we will conduct transcriptomic analyses on PFAS-exposed macrophage-like THP-1 cells to identify differentially expressed gene networks. Understanding how PFAS affect innate immunity will help us better understand how PFAS exposure can alter an organism’s ability to resist pathogens in its environment as well as cancer.

**1.02.V Advancements in Aquatic and Wildlife Immunotoxicology: Innovative Approaches to Identifying Adverse Outcomes**

**1.02.V-01 Contaminant Exposure and Gene Transcription in Baltic and Greenlandic Ringed Seal Tissues**
***Joy Ometere Boyi***[1]*, Christian Sonne*[2]*, Jens Søndergaard*[2]*, Sara Persson*[3]*, Frank Riget*[2]*, Cynthia A. de Wit*[4]*, Ursula Siebert*[1] *and Kristina Lehnert*[1]*, (1) University of Veterinary Medicine Hannover, Germany, (2) Aarhus University, Denmark, (3) Swedish Museum of Natural History, Sweden, (4)Stockholm University, Sweden*

Ringed seals are recovering in the Baltic Sea after decades of hunting pressure and contaminant exposure, while still sustainably harvested in Greenland by subsistence hunters. Consequences of anthropogenic stressors such as contaminant exposure and increasing temperatures continue to have deleterious effects on both populations. To assess the impacts of environmental stress on ringed seals from different geographic regions, selected persistent organic pollutants (POPs) were measured in blubber and liver of Baltic (n = 15), and total mercury measured in liver of Baltic (n = 15) and Greenlandic (n = 16) ringed seals. In addition, mRNA transcript levels of the health- and pollutant-related genes; aryl hydrocarbon nuclear translocator (*ARNT*), peroxisome proliferator-activated receptor alpha (*PPARα*), thyroid receptor alpha (*TRα*), estrogen receptor alpha (*ESR1*) and heat shock protein 70 (*HSP70*) were quantified in blood and blubber using RT-qPCR. The mRNA transcript levels did not vary between sexes or age classes and the highest transcript levels of endocrine and metabolic markers were observed in blubber. Cytokines *IL-10* and *IL-2* as infectious disease markers showed low transcript levels in Greenlandic seals. Significantly higher levels of xenobiotic- and endocrine-related gene transcription (*ARNT*, *PPARα, TRα* and *ESR1*) were observed in Baltic compared to Greenlandic ringed seals. Transcript levels of *ARNT*, *HSP70* and *TRα* correlated with levels of POPs, and *TRα* was related to mercury concentration in liver of Baltic ringed seals. No significant correlation between mRNA transcript levels and mercury concentration in ringed seals from Greenland was observed. However, higher *HSP70* levels in the Greenlandic ringed seals compared to the Baltic may indicate some elevated stress due to other environmental stressors. Our results reflect a continued high PCB exposure in Baltic ringed seals, and contaminant-associated health effects in contrast to more pristine waters around Greenland. Gene transcript profiles from blubber highlight the value of minimally invasive sampling for assessing health endpoints in free ranging marine wildlife. In conclusion, contaminant exposure and environmental conditions influence the health of ringed seals, and gene transcript profiles are good indicators of early on-set effects from combined environmental stressors. The results are important for timely conservation and management of ringed seals in the Baltic and around Greenland.

## 1.03.P Adverse Outcome pathways - Development and Applications

### 1.03.P-Th001 AOP-Wiki Release 2.5 - What's new with the Adverse Outcome Pathway Framework?

***Daniel L. Villeneuve****, U.S. Environmental Protection Agency*

The adverse outcome pathway (AOP) framework and AOP-Wiki are designed to capture and disseminate synthesized knowledge and evidence concerning causal relationships between stressor-induced perturbation of biological systems at the molecular, biochemical, and cellular levels of biological organization and consequent adverse effects of regulatory and/or risk management significance. While the underlying philosophy and principles of AOP development have remained static, details concerning the pertinent information to collect, overall approach for organizing and presenting the information in the AOP-Wiki, and guidance continues to evolve based on experience and feedback from the AOP community of practice. This presentation summarizes some of the key changes to the AOP framework associated AOP-Wiki release 2.5 and corresponding updates to the AOP Developers' Handbook. With release of AOP-Wiki 2.5, AOP developers are now requested to provide information concerning their AOP development strategy and methods employed for evidence collection in support of key event relationships. Listing and searchability by stressors have been deemphasized, in keeping with the principle that AOPs are not stressor-specific. However, identification of prototypical stressors that can serve as useful reference compounds or positive controls for activity along a given AOP is encouraged. Templates for capturing empirical support for key event relationships as well as documenting known modulating factors have been added. Direct links to third party tools to aid both development, visualization, and/or use of AOPs (e.g., Wiki-Kaptis; AOP helpFinder) have been added. Finally, a more easily accessible and interactive version of the AOP Developers' Handbook, along with appropriate version labelling for AOPs initiated under previous versus updated guidance documents have been implemented. These changes lay the foundation for a transition to a new AOP-Wiki data model that will further enhance the fit-for-purpose use of information captured in the AOP-Wiki. *The contents of this abstract reflect the contributions of multiple sub-*

*groups of the OECD Extended Advisory Group on Molecular Screening and Toxicogenomics, but do not constitute or necessarily reflect official policy of the OECD or any of its member countries or organizations.*

**1.03.P-Th002 Elucidating the mechanism through which low-dose neonicotinoid and pyrethroid insecticides disrupt insect ecdysis**

***Niranjana Krishnan***[1]*, Cassandra Gorman*[2]*, Steven Bradbury*[1] *and Russell Jurenka*[1]*, (1) Iowa State University, (2) Missouri University of Science and Technology*

Neonicotinoids and pyrethroids, two commonly used neurotoxic insecticides, are effective against multiple insect species. Neonicotinoids mimic the neurotransmitter acetylcholine while pyrethroids disrupt the function of voltage-gated sodium channels. Additionally, type II pyrethroids can antagonize GABA signaling. At high doses, both classes of insecticides cause neuronal overstimulation, paralyses, and death. The current presentation focuses on their low-dose effects, specifically, their interference with the insect ecdysial process (i.e., shedding of the old cuticle).

Lepidopteran (moths and butterflies) larvae and coleopteran (beetles) pupae topically exposed to low doses of neonicotinoids and type II pyrethroids fail to undergo pupal and adult ecdysis, respectively. Species from both orders initiated but did not complete ecdysis and had unexpanded or unextended adult appendages. Experiments with type I pyrethroids, which do not antagonize GABA signaling, produced very low rates of arrested pupal and adult ecdysis. These observations, along with studies that provide the timing of key ecdysial events, suggest that neonicotinoids and type II pyrethroids may be interfering with the function of crustacean cardioactive peptide (CCAP) neurons, which are responsible for successful ecdysis and protracted appendages.

Studies to date have elucidated the role of neuroendocrine hormones in insect ecdysis; the role of excitatory and inhibitory neurotransmitters is less understood. Our results indicate that acetylcholine and GABA can directly or indirectly regulate CCAP neurons, either by preventing their excitability or prolonging their inhibition. These effects on CCAP neurons could hinder the timely release of CCAP and other neuropeptides that regulate ecdysis. Full elucidation of the mechanism through which neonicotinoids and type II pyrethroids disrupt insect ecdysis will provide the means to model low-dose effects and assess interspecies variability. Findings from the research will have implications in insect pest management and non-target risk assessment.

**1.03.P-Th003 Defining the Biologically Plausible Taxonomic Domain of Applicability Can Enhance the Utility of an Adverse Outcome Pathway for Understanding Apis and Non-Apis Bee Health**

***Marissa Jensen***[1]*, Donovan Blatz*[2] *and Carlie LaLone*[2]*, (1) University of Minnesota, (2) U.S. Environmental Protection Agency*

*Apis* bee health has gained significant attention due to the increases in colony death and failure. Chemical stressors, such as neonicotinoid insecticides, along with non-chemical stressors contribute to impact on colonies. In addition to the concern surrounding *Apis* bee populations, there are concerns regarding non-*Apis* bee populations, such as bumble bees and solitary bees. To begin to better understand the possible linkages between stressors and declines in *Apis mellifera* bee health, an adverse outcome pathway (AOP) network was previously published describing the weight of evidence connecting activation of the nicotinic acetylcholine receptor to an adverse outcome of colony death/failure. Because this network was developed focusing only on *Apis mellifera*, there are uncertainties regarding extrapolation to other bee species. Defining the taxonomic domain of applicability (tDOA) of an AOP is important for regulatory decision-making, especially when considering the protection of untested bee species. Because protein structural and/or functional conservation are two components that can be considered when defining tDOA, bioinformatics tools such as the U.S. Environmental Protection Agency's Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) can provide lines of evidence of structural conservation across species and therefore help inform biologically plausible tDOA descriptions. Results show evidence of structural conservation across many *Apis* and non-*Apis* bees in early key events, however, downstream apical key events are expected to differ across bee species, depending on characteristics like colony structure. Even though early key events may be linked to different outcomes for some species, understanding how broadly the early key event biology can be extrapolated is useful for mitigation strategies, especially for bee species that are normally untested. A decision tree can also be applied to understand whether it is plausible that other bee species could be included in the tDOA. Expanding tDOA descriptions for key events and key event relationships in this AOP can increase its utility in decision-making and highlights knowledge gaps to guide further research. *The views expressed in this work are those of the authors and do not necessarily reflect the views or policies of the US EPA.*

**1.03.P-Th005 Avian-Specific Evidence for Endocrine Disruption Adverse Outcome Pathways from Chicken Embryos Exposed to Bisphenol A and Ethinylestradiol**

***Tasnia Sharin***[1]*, Kim Williams*[2]*, Doug Crump*[2] *and Jason O'Brien*[2]*, (1) University of Ottawa, Canada, (2)Environment and Climate Change Canada*

Several adverse outcome pathways (AOPs) that document how endocrine disrupting compounds (EDCs) can interfere with vitellogenin synthesis, leading to reduced fecundity and decreased population size have been described in the AOP-wiki. However, these AOPs are primarily described for fish. There are limited data to support the relevance of these AOPs for avian species. Here, we employed a chicken embryo model to generate data on different endpoints associated with estrogen response following exposure to two EDCs, bisphenol A (BPA; 5 to 100µg/g) and ethinylestradiol (EE2; 0.5 to 50µg/g). Embryos were exposed via egg injection prior to incubation and data were collected at two developmental stages, mid-incubation (embryonic day [ED] 11) and term (ED20). Embryo viability was determined at ED11, changes in gonad morphology were determined at ED20, and expression of hepatic estrogen-responsive genes and plasma vitellogenin were measured at both time points. Both BPA and EE2 resulted in modulation of estrogen-responsive genes, including upregulation of vitellogenin, in mid-incubation embryos. The magnitude of change in gene expression was greater in mid-incubation embryos compared to term. BPA (50µg/g) increased plasma vitellogenin concentration in term embryos, while EE2 (0.5 to 25µg/g) increased plasma vitellogenin at both developmental stages. Gonad histological observations indicated accumulation of proteinaceous fluid and loss of seminiferous tubules in males after 50µg/g BPA exposure. Male embryos treated with EE2 (0.5 and 25µg/g) had loss of tubules and increased cortex thickness similar to ovaries. The estimated LD50 values for BPA and EE2 at mid-incubation were 100.13µg/g and 71.93µg/g. In summary, the findings suggest that BPA and EE2 upregulated vitellogenin mRNA expression, which is followed by increased plasma vitellogenin levels and impairments in gonadal development. These results contribute avian-specific evidence for endocrine disruption AOPs that describe the relationship between disrupted vitellogenin synthesis and impaired reproduction.

**1.03.P-Th006 AOP 310: Embryonic activation of the Ah Receptor in fishes leading to reproductive failure, via epigenetic down-regulation of GnRHR**

***Cameron Hunter Collins***[1]*, Steve Wiseman*[2] *and Jon Doering*[1]*, (1) Louisiana State University, (2) University of Lethbridge, Canada*

This adverse outcome pathway (AOP) describes the linkage between activation of the aryl hydrocarbon (Ah) receptor in embryos of fishes and reproductive failure at adulthood. The Ah receptor is expressed by at least 3 hours post-fertilization. Various environmental contaminants with a planar structure can activate the Ah receptor, including polycyclic aromatic hydrocarbons (PAHs) and dioxin-like compounds (DLCs). Immediately following activation of the Ah receptor in embryos is an up-regulation of DNA methyltransferases (DNMTs) which serve to manage DNA methylation. An increase in expression of DNMTs results in the hypermethylation of the promoter region of genes which subsequently can cause the life-long and transgenerational down-regulation in expression. Promoter region hypermethylation can occur for genes involved in reproduction. Down-regulation in expression of reproductive genes in female fishes, such as gonadotropin releasing hormone receptors (GnRHRs), aromatase (CYP19), or cholesterol side-chain cleavage enzyme (CYP11), can result in decreased synthesis of sex steroids, such as estrogen (E2). In female fish, E2 activates the estrogen receptor (ER) which drives production of the egg-yolk precursor protein, vitellogenin (VTG). Less E2 reduces the production of vitellogenin (VTG) which decreases the ability of female fish to produce viable eggs. Linkages in this AOP have been demonstrated through studies of zebrafish (Danio rerio) exposed to the PAH, benzo[a]pyrene. However, quantitative response-response relationships and relevance to other fishes are completely unknown. A quantitative understanding of the linkages from initial exposure of embryos to reproductive failure at adulthood would allow for this AOP to be referred to when conducting ecological risk assessments, and ultimately guide more objective risk assessments of the sublethal and long-term impacts of exposure to chemicals that activate the Ah receptor.

**1.03.P-Th007 Development of neurobehavior AOP: Comparisons of multiple endpoints and fish species after exposure to neurotoxicants**

*Janice Albers[1], **Cheryl A. Murphy**[1], Natalia Reyero[2], Michael Carvan[3] and Adam Thrash[4], (1) Michigan State University, (2) U.S. Army Engineer Research and Development Center, (3) University of Wisconsin, Milwaukee, (5) Mississippi State University*

Understanding the ecologically relevant sublethal impacts on fish populations from human pollution involves interpreting complex biological information collected from suborganismal and organismal levels of biological organization.  The Adverse Outcome Pathway framework (AOP) is proposed as a way to organize and predict impacts on suborganismal processes, ecologically relevant species from model species and different chemicals.  AOPs could potentially expand the application of current studies that would ultimately decrease the amount of laboratory testing performed in toxicology.  AOP assumptions were examined in this study; specifically, whether AOPs could predict adverse effects just from perturbation of biologically processes, if AOPs could extrapolate to other species from laboratory species, and to simplify toxicity endpoints by using measurable correlates in key events. Our study collected endpoints at multiple biological levels on three fish species after expose to two neurotoxicants and determined altered brain gene expression, fish behavior, and modeled young-of-the-year (YOY) cohort survival and growth.  In conducting this study, we discovered that what is needed is critical attention to chemical exposures regimes, animal husbandry, sequenced genomes, and analytical techniques that combine a diverse suite of biological endpoints.  Preliminary results suggest little common responses for both chemicals and all fish species. However, there may be consistency with some gene expression and behavior endpoints within species and chemicals.  Results from this study suggest limitations to predicting sublethal impacts across the species and chemicals in this study using the AOP framework, but overcoming identified limitations and further development in critical areas may help.

**1.04 Ecotoxicology and Risk Assessment of Reptiles and Amphibians**

**1.04.T-01 Relationship Between Serum Thyroid Hormone Metabolites and Gene Expression Biomarkers in the Back Skin During Rana [Lithobates] catesbeiana Tadpole Metamorphosis**

*Rikke Poulsen[1], Shireen H. Jackman[2], **Caren C. Helbing**[2] and Martin Hansen[1], (1) Aarhus University, Denmark, (2) University of Victoria, Canada*

Anuran metamorphosis is characterized by profound morphological changes including remodeling of tissues and organs. This transition is initiated by thyroid hormones (THs), however, currently, the knowledge of baseline levels of THs during metamorphosis relies on outdated methods and limited sample replication. Applying state-of-the-art isotope-dilution liquid chromatography tandem mass spectrometry, the current study presents a quantification of 11 thyroid hormones and their associated metabolites in the serum of *Rana [Lithobates] catesbeiana* tadpoles from seven distinct postembryonic stages from premetamorphosis (Gosner stage 31) through to a juvenile frog (Gosner stage 46; n=5-10).

In addition to the TH profile, we also analyzed the abundance of TH-relevant gene transcripts (*thra*, *thrb*, and *thibz*) in back skin of the same animals. As expected THs increased during metamorphosis and reached the highest concentration at metamorphic climax (Gosner stage 44). While *thra* transcript levels remained consistent, *thrb* and *thibz* transcript levels began increasing at Gosner stage 40 reaching a maximum at stage 41.

This exemplifies the exquisite timing of events during metamorphosis as the synthesis of the receptor needs to be ready for the peak in TH concentration.

This finding also underlines the sensitivity of the developmental process, and it is, indeed, well-known that external factors such as anthropogenic chemicals can disrupt anuran metamorphosis. Hence, as a second aim of the study, we set out to find additional biomarkers of metamorphosis, which can aid future investigations of developmental disruption. Using a nanoLC-Orbitrap system an untargeted analysis workflow was applied. Among 6,062 detected endogenous metabolites, 421 showed significant metamorphosis-dependent concentration dynamics like the TH-related metabolites. Among these potential biomarkers were several prostaglandins and other eicosanoids, some steroid hormones, and several carnitines. These data provide a necessary context for interpreting TH-mediated developmental processes and provides a solid foundation from which alternative biomarkers of metamorphosis may be chosen as feasible endpoints in future risk assessment of chemicals and their potency for causing developmental toxicity.

**1.04.T-02 Development of a native amphibian toxicology model in an outdoor system**

***Shauni Windle***[1]*, Scott McMurry*[1]*, Jonathan Maul*[2]*, Richard Brain*[2]*, Jeffrey C. Wolf*[3] *and Jason B. Belden*[1]*, (1) Oklahoma State University, (2) Syngenta Crop Protection, (3) EPL, Inc.*

Over the decades, the field of ecotoxicology has utilized both indoor laboratory designs as well as outdoor designs to assess the effect of toxic compounds on amphibians. Furthermore, various model and native species have been used to illustrate effects across taxa. We propose the use of a relatively unused species, *Acris blanchardi*, for the purpose of assessing toxicological effects after chronic exposure. As a short-lived native species, *A. blanchardi* not only provides the opportunity to assess general developmental effects but also reproductive effects due its uniquely fast maturity. Within a single field season (approximately 90 days), *A. blanchardi* hatch and reach sexual maturity before overwintering. This life attribute allows for assessment of reproductive toxicity in a few months of developmental exposure. Furthermore, due to their abundance in numerous North American areas, *A. blanchardi* tadpoles are easy to procure from breeding adults. The objective of our work with *A. blanchardi* was to develop a model where we tested *A. blanchardi* rearing, toxicity exposure, and resulting responses. Using 17β-estradiol (E2) as an endocrine disrupting compound (EDC), we developed and tested a toxicity assay with *A. blanchardi* in an outdoor system. Use of an outdoor system allowed for an additional 60 days of post-metamorphosis maturation in a terrestrial setting. In addition to experimental animals, we captured and assessed reproductive maturity of wild *A. blanchardi* over three time points and from three different ponds. Wild animals were collected in the spring at emergence, summer during active breeding, and in the fall immediately before overwintering. Due to the short lifespan of *A. blanchardi,* fall animals were exclusively newly metamorphosed individuals and were most similar in life stage to experimental animals. We were able to not only demonstrate the ability to test this species from egg to sexually mature in a single season but also recovered valuable data regarding the effect of a well-known EDC on *A. blanchardi.* After chronic exposure to E2, we found that *A. blanchardi* were relatively insensitive to concentrations shown to induce complete feminization in other amphibian species. However, we believe that our overall results illustrate positive potential for this species to be used as an amphibian model in future toxicity studies.

**1.04.T-03 Effects of per-/polyfluoroalkyl substances (PFAS) on the hematology and immune response of Northern watersnakes (Nerodia sipedon)**

***David Lee Haskins***[1,2]*, Melissa E. Lech*[1]*, Devin Kyle Jones*[1]*, Kathryn A. Quinlin*[1]*, Matthew T. Hamilton*[1]*, Maggie A. Wigren*[1]*, Elizabeth G. Hoffman*[1]*, Youn Jeong Choi*[1]*, Kristina Meichner*[3]*, Linda S. Lee*[1] *and Jason T. Hoverman*[1]*, (1) Purdue University, (2) U.S. Geological Survey, (3)University of Georgia*

Per- and polyfluoroalkyl substances (PFAS) are a widely used family of chemicals that are of concern due to their persistence and potential accumulation in both humans and wildlife. However, our understanding of adverse effects is lacking for most wildlife species, especially reptiles. Moreover, most studies with wildlife have been acute toxicity trials with model species using a single PFAS. To better inform ecological risk assessment, we require studies that examine sublethal exposures to PFAS mixtures that mimic environmental exposures in non-model wildlife species. In this study, we exposed 71 juvenile Northern watersnakes (*Nerodia sipedon*) to one of four PFAS treatments: control (n = 18), perfluorooctanesulfonate (PFOS, 3000 ppb; n = 17), perfluorohexanesulfonate (PFHxS, 70 ppb; n = 18), and a PFAS mixture group (n = 18). The PFAS mixture was composed of 3000 ppb PFOS, 70 ppb PFHxS, 0.4 ppb perfluorooctanoic acid (PFOA), 0.4 ppb perfluorohexanoic acid (PFHxA), and 0.3 ppb perfluoropentanoic acid (PFPeA). Watersnakes were exposed to PFAS through diet (PFAS-spiked fathead minnows), and treatment concentrations were based on PFAS concentrations reported in similar prey items from sites heavily impacted by PFAS contamination. To evaluate the hematologic and immune responses of *N. sipedon* to PFAS, we assessed differences in packed cell volume, total solids, bacterial killing assays, and white blood cell differentials at the end of our study. Preliminary analyses suggest significant negative impacts of PFAS exposure on the hematology (e.g., packed cell volume, total solids) of juvenile watersnakes ($t = 2.65 – 3.34$, $p = 0.001 – 0.049$). We are currently processing other samples (bacterial killing assays and white blood cell differentials) and performing further analyses to identify potential effects of PFAS exposure in watersnakes. Our findings will provide a foundation for understanding how these ubiquitous contaminants may influence the health of squamates.

**1.04.T-04 Evaluation of anticoagulant rodenticides, warfarin and diphacinone sensitivity in sea turtles**
***Shouta M.M. Nakayama***[1]*, Yoshiya Yamamura*[1]*, Satomi Kondo*[2]*, Chiyo Kitayama*[2]*, Takahiro Ichise*[1]*, Yoshinori Ikenaka*[1] *and Mayumi Ishizuka*[1]*, (1) Hokkaido University, Japan, (2) Everlasting Nature of Asia (ELNA), Japan*
Although anticoagulant rodenticides (ARs) are effectively used for the control of invasive rodents, nontarget species are also frequently exposed to ARs and secondary poisonings occur widely. However, little data is available on the effects of ARs, especially on marine organisms. To evaluate the effects of ARs on marine wildlife, we chose green sea turtles (Chelonia mydas), which are one of the most common marine organisms around the Ogasawara islands, as our primary study species. The sensitivity of these turtles to ARs was assessed using both in vivo and in vitro approaches. We administered 4 mg/kg of warfarin sodium either orally or intravenously to juvenile green sea turtles. The turtles exhibited slow pharmacokinetics, and prolongation of prothrombin time (PT) was observed only with intravenous warfarin administration. We also administered 4 mg/kg of diphacinone intravenously. Concentration of diphacinone in green turtle plasma did not decrease and about 50% of Cmax value was still observed after 216h of administration of the drug. About two-fold prolongation of PT was observed 9 days after dosing compared to before dosing, suggesting that diphacinone exposure causes hematologic toxicity in green turtles. We also conducted an in vitro investigation using liver microsomes from green sea turtles, and two other turtle species (softshell turtle and red-eared slider) and rats. The cytochrome P450 metabolic activity in the liver of green sea turtles was lower than in rats. Additionally, vitamin K epoxide reductase (VKOR), which is the target enzyme of ARs, was inhibited by warfarin in the turtles at lower concentration levels than in rats. These data indicate that turtles may be more sensitive to ARs than rats. We expect that these findings will be helpful for sea turtle conservation following accidental AR-broadcast incidents. In the future, by focusing on other factors that determine the strength of toxicity, such as the binding rate of serum albumin to rodenticides (ratio of free diphacinone concentration), we intend to conduct a more detailed evaluation and establish and apply the PT measurement method as a screening test to evaluate the exposure status of wild sea turtles to rodenticides.

**1.04.T-05 Acute and Chronic Effect of Current-use Fungicides on Survival, Growth, and Development of Wetland-breeding Amphibian Larvae**
***Andrew Patrick Hopkins*** *and Jason T. Hoverman, Purdue University*
Fungicides are increasingly applied to a wide range of commercial crops globally. Despite their widespread usage, the ecotoxicity of fungicide is understudied relative to other pesticide types. Furthermore, studies examining the effects of fungicides on amphibians make up a small portion of the current research efforts. We examined the acute and chronic effects of two current use fungicides, pyraclostrobin and chlorothalonil, on

larval amphibians that would be endemic to the ponds and areas most likely to be contaminated with fungicides. For six species, calculated LC50 values for acute toxicity ranged from 4 – 21 µg/L and 15 – 50 µg/L for pyraclostrobin and chlorothalonil, respectively. These values are close in magnitude to the expected environmental concentration for the fungicides suggesting that environmental exposures could lead to direct mortality in these species. To explore chronic effects of fungicide exposure, we conducted studies with three species that were exposed to 0, 0.15, 1.5, 4.5, 7.5, or 10 µg/L of pyraclostrobin, all of which are within the expected environmental concentration (EEC). We used a fast-developing species, *Rana sylvatica*, a species with intermediate developmental rate, *Hyla versicolor*, and a slow developing species, *Rana catesbeiana* to examine effects on larval growth and development. For the fast-developing species (*R. sylvatica*) pyraclostrobin (7.5 and 10 µg/L) exposure delayed time to metamorphosis and caused larger snout-vent length (SVL) and mass. While larger size can improve fitness post-metamorphosis, delayed metamorphosis can impair the ability of this species to escape their ephemeral ponds that dry each year. There were no effects detected in the other two species. Collectively, our data suggest that some fungicides can pose acute and chronic toxicity risks to amphibians at or below their EEC.

**1.04.T-06 Dirtier and Sicker: Site- and Individual-Level Contamination Affects Infection Prevalence of an Emerging Infectious Disease of Amphibians**

***Kelly L. Smalling***[1]*, Brittany Mosher*[2]*, Luke R. Iwanowicz*[1]*, Michelle L. Hladik*[1]*, Keith Loftin*[1]*, Adam Boehlke*[1]*, Carly Muletz-Wolz*[3] *and Evan Grant*[1]*, (1) U.S. Geological Survey, (2) University of Vermont, (3) Smithsonian National Zoo and Conservation Biology Institute*

Emerging infectious disease outbreaks are one of multiple stressors responsible for amphibian declines globally. In the northeastern United States, ranaviral diseases are prevalent in amphibians and other ectothermic species, but there is still uncertainty as to whether their presence is leading to population declines. Further, there is uncertainty surrounding the potential interactions among fungal disease infection prevalence in free-ranging amphibians and habitat degradation (co-occurrence of anthropogenic stressors). The current study was designed to provide field-based estimates of the relationship between amphibian disease and anthropogenic stressors. We visited 40 wetlands across three Department of the Interior protected areas in the northeast, estimated the prevalence of ranavirus among populations of larval wood frogs and spotted salamanders, and assessed chemical and biological stressors in wetland habitats and larval amphibians using a suite of selected bioassays, screening tools and chemical analyses. Estimated ranavirus occupancy ranged from 0.27 to 0.55 with considerable variation within protected area. Of the stressors evaluated, ranavirus prevalence was strongly and positively related to concentrations of metalloestrogens (metals that have the potential to bind to the estrogen receptor) and total metals in wetland sediments and weakly and negatively related to total pesticide concentrations (sum of all pesticides detected) in larval amphibians. These results can be used by land managers to refine habitat assessments to include such environmental factors with the potential to influence disease susceptibility.

**1.04.T-07 Subacute effects of microcystin on larval and embryonic Rana clamitans hatch success, growth, and survival**

***Brenna Friday****, Judy Westrick and Donna Kashian, Wayne State University*

Many chronic and acute harmful effects that cyanotoxins pose to humans, fish, and aquatic macroinvertebrates are known following a plethora of experiments in recent years. Unfortunately, many of these studies have overlooked developing amphibians. Yet this group that may be exposed to high levels of cyanotoxins and particularly sensitive at this young life history stage. To address this gap, I designed a series of laboratory experiments to evaluate hatch success, growth, and survival in larval green frogs (*Rana clamitans*) exposed to microcystin-LR (MCLR), a common toxin synthesized by cyanobacteria. In July 2021, frog eggs were collected from a natural pond in Michigan, hatched in lab, and maintained for 6 weeks before the first experiment. Animals were exposed in water to 100 µg MCLR/L for 18 days and monitored daily for changes in growth, weight, and survival. Two different MCLR treatments were used to explore biochemical mechanisms of toxicity: 1) synthetic MCLR powder or 2) MCLR from lysed cyanobacterial cells collected from a bloom in Lake Erie. Five tanks each housing 3 tadpoles were randomly assigned to control, synthetic toxin, and lake toxin groups ($n = 45$). Survivorship ($p=1.00$), weight gain ($p = 0.16$), and growth as measured by increase in total length ($p = 0.14$) did not differ among treatments over the 18 days. The inability to detect lethal or sublethal impacts of MCLR to larval *R. clamitans* suggests they may be more tolerant of harmful algae blooms than many other aquatic species. This interaction will be explored further in July 2022 when field collected *R. clamitans* eggs will be exposed to 0-100 µg MCLR/L to measure how embryonic toxin exposure impacts egg development. If tadpoles successfully develop following embryonic exposure, additional toxin susceptibility studies will explore how secondary toxin exposure influences feeding behavior, growth, and development in *R. clamitans* tadpoles. Together, these experiments provide insights into how harmful algae blooms may impact amphibian population dynamics in aquatic ecosystems.

**1.04.T-08 Discussion on Climate Change and Ecotoxicology for Herpetofauna**
***Celine A. Godard***[1] *and Paula F.P. Henry*[2]*, (1) Texas Tech University, (2) U.S. Geological Survey*
During the discussion session, presenters and conference attendees are welcome to share their views on the future of herpetofauna in a changing world and the future of herpetofauna toxicology in general. There will also be an opportunity to share suggestions on increasing the scope of herpetofauna toxicology research, successfully recruiting new generations of scientists, ensuring funding agencies support our field, increasing international collaborations, etc. It would be fruitful for the 3 groups, government, academia and business, to meet and see how we might be able to interact with each other in these efforts.

**1.04.P Ecotoxicology and Risk Assessment of Reptiles and Amphibians**

**1.04.P-Tu003 Rural vs. Urban Amphibians: Assessing Abundance, Body Condition, and Dietary Toxicant Exposure**
***Amalia M Conner****, Christopher J. Salice, Taylor S Anderson and Alexander Pellegrini, Towson University*
Biodiversity is critically important to the health of ecosystems as it is positively related to higher organismal fitness, greater resistance to stress and disturbance, and improved delivery of ecosystem services to humans. Increased urbanization threatens biodiversity of many ecosystems because of associated increases in habitat loss and fragmentation, pollution, and introduced diseases. Although many taxa are impacted by urbanization, amphibians are considered one of the most sensitive and imperiled due to their permeable skin, presence in aquatic habitats, and low dispersal capabilities. This research aims to improve our understanding of how amphibian populations vary across an urban-rural gradient. We expect to see lower abundance and poorer body condition of amphibians in urban sites relative to rural sites. The target species in this research included the red-backed salamander (*Plethodon cinereus*), the northern two-lined salamander (*Eurycea bislineata*), the eastern newt (*Nothophthalamus viridescens*), the green frog (*Lithobates clamitans*), the American toad (*Anaxyrus americanus*) which are common to Maryland. Body condition and abundance data were collected to assess the overall health of salamanders present at sampling sites. Additionally, we will measure per- and polyfluoroalkyl substances (PFAS) in terrestrial invertebrate samples as an overall indicator of dietary exposure of amphibians to anthropogenic chemical contamination. Liquid Chromatography with tandem mass spectrometry (LC-MS-MS) will be used to quantify PFAS concentrations within sampled individual prey items. Results from this research will add to our understanding of the abundance and potential chemical impacts to amphibians in Central Maryland and will be useful in developing conservation strategies for this imperiled taxon.

**1.04.P-Tu004 Assessing Methylmercury Exposure of Plethodontid Salamanders in New York State**
***Jeremiah Harvey Tennant***[1]*, Bradley J. Cosentino*[2]*, Kristen M. Brubaker*[2]*, Lisa B. Cleckner*[2] *and N. Roxanna Razavi*[1]*, (1) SUNY College of Environmental Science and Forestry, (2) Hobart and William Smith Colleges*
Salamanders serve as bioindicators of mercury in both terrestrial and aquatic habitats. They play an integral part in the food web, being both a predator of many invertebrates and prey to many higher vertebrates. The

objectives of this study were to determine if diet (as measured by trophic position) is a driver of methylmercury (MeHg) exposure, determine the effect of habitat use on MeHg bioaccumulation, and compare MeHg concentrations between a mercury hotspot (Adirondack Mountains) and reference site (Finger Lakes National Forest) in New York State. Tail samples were collected from one terrestrial salamander species (*Plethodon cinereus*) and two semiaquatic species (*Eurycea bislineata*and *Desmognathus fuscus*) and analyzed for MeHg content. Trophic position ($\delta^{15}$N) did not predict MeHg accumulation in the two morphs of *P. cinereus* or *E. bislineata*, but it did in *D. fuscus*. However, habitat did affect MeHg exposure, with semiaquatic salamanders having higher MeHg concentrations than terrestrial salamanders. Carbon source ($\delta^{13}$C) was a significant predictor of MeHg in the terrestrial species but not in the semiaquatic species. The semiaquatic species had significantly lower $\delta^{13}$C values, and significantly higher $\delta^{15}$N and MeHg compared to the terrestrial species. Surprisingly, the Adirondack Mountains did not have higher MeHg concentrations compared to the Finger Lakes National Forest. This work establishes baseline MeHg data for salamanders in these two regions for future assessments of changes to mercury deposition and bioaccumulation in forests of New York State.

**1.04.P-Tu005 Detecting the Emerging Contaminant Methamphetamine in Apex predator Alligator mississippiensis**

***Terri Cox****, Sarah Nash, Brandon Gross, Seenivasan Subbiavasan, Jordan Crago and Lou Densmore, Texas Tech University*

There are many environmental contaminants that have persisted in the ecosystem for decades. In fact, many pesticides such as those belonging to the DDT family and polychlorinated biphenyls (PCBs) continue as legacy contaminants due to their unique longevity in biota for many decades after contamination. Additionally, numerous studies have demonstrated the detrimental effects of pharmaceuticals on aquatic species such as fish, amphibians, and crustaceans. In fact, there is a new class of emerging contaminant that has been detected in aquatic systems and organisms such as the previously mention: illicit drugs. Alligator mississippiensis is a prolific opportunistic predator, is semi-aquatic, and consumes prey that have been known to contain such contaminants as pesticides, pharmaceuticals, and illicit drugs, we predict that such emerging contaminants can be detected in apex predators such as A. mississippiensis. Given that areas of high drug trafficking and high use such as the Houston metropolitan area overlap with organisms such as A. mississippiensis is, it is predicted that such contaminants can be detected in semi-aquatic organisms. Conversely, in areas such as wildlife centers far removed from urbanized area such as the Rockefeller Wildlife Refuge in Grand Chenier, LA. it is predicted there will be little to no contaminants such as amphetamine and/or its metabolites. This study focus on the detection of the amphetamine class of drugs such as amphetamine, MDA, MDEA, MDMA, and methamphetamine in the apex predatory A. mississippiensis. Results from this study indicate that amphetamine was detected in alligator adipose, liver, and scute tissue. Given the ubiquity of this contaminant across tissue types, this study present data examining the difference in concentration across size classes such as juvenile, sub-adult and adult, and gender.

**1.04.P-Tu006 Validation of an Appropriate Healthy Diet for Xenopus Laevis Used in Endocrine Studies**

***Emma Danby***[1]*, Dominic Sacker*[1,2] *and Lauren Jones*[1]*, (1) Labcorp Early Development Laboratories Limited, United Kingdom, (2) Covance, United Kingdom*

The Amphibian Metamorphosis Assay (AMA:OECD 231) recommends a tadpole diet of the commercial frog food Sera Micron. When used on these studies, the frogs did develop well but inconsistent and with several cases of scoliosis (curvature of the spine). In many cases the scoliosis was considered so severe that the animals were terminated on welfare grounds. In order to reduce animal waste and have a much more consistent growth and development in the frogs, we trialed various different diets and looked at many different endpoints assessed.

Assessments of survival and general observations were monitored daily throughout the test. On Day 7 of the test, a sub-sample of tadpoles from each replicate were assessed for body weight, normalized left hind limb length, snout to vent length (SVL) and developmental stage. On termination of the test at Day 21, body weight, normalized left hind limb length, SVL and developmental stage was performed on remaining tadpoles and finally a sub-sample from each replicate was sent for histopathological examination of the thyroid.

Looking at all these endpoints together, we have successfully validated a new diet that had no impact on developmental rate or thyroid function but did reduce scoliosis and have a positive result of producing consistent developmental growth between batches of tadpoles.

**1.04.P-Tu007 Effects of Perfluoroalkyl Substances (PFAS) On Amphibian Body Condition: Is Altered Lipid Metabolism the Driver?**

***Anna Grace Bushong**, Tyler D. Hoskins and Marisol S. Sepulveda, Purdue University*

Per- and polyfluoroalkyl substances (PFAS) are globally distributed, emerging contaminants with widespread use in commercial and consumer products. Amphibians are susceptible to sublethal effects of PFAS exposure and are regularly exposed in the field. In amphibians exposed to PFAS, body condition, measured using the scaled mass index (SMI), is often affected. *In vitro* and rodent studies have demonstrated that PFAS can alter lipid metabolism via binding to peroxisome proliferated alpha receptors (PPARs) and associated dysregulation of signaling cascades for lipid homeostasis. However, whether PPAR-mediated changes in fat stores can explain previously observed PFAS-mediated changes in amphibian SMI remains unexplored. Because lipids are a primary fuel for anuran metamorphosis and reproduction, understanding whether altered lipid metabolism can explain effects on SMI is critical. Further, SMI has been shown to correlate with lipid stores in several species of amphibians, but effects of PFAS exposure and the relationship between SMI and lipid stores have not been investigated in the amphibian model, *Xenopus laevis.* Our central objective was to determine if and how PFAS exposure affects growth, development, SMI, lipid deposition, or PPAR-mediated signaling cascades in *X. laevis*. We chronically exposed *X. laevis* to environmentally relevant concentrations of perfluorooctanesulfonic acid (PFOS, 0.5 mg/L), perfluorohexanesulfonic acid (PFHxS, 0.5 mg/L), perfluorooctanoic acid (PFOA, 0.5 mg/L), perfluorohexanoic acid (PFHxA, 0.5 mg/L), a binary mixture of PFOS and PFHxS (0.5 mg/L of each), or a no PFAS control, beginning at Nieuwkoop Faber (NF) stage 52. We sampled animals at NF 58, 62, and 66 and measured length, mass, SMI, and time-to-stage. We also quantified carcass lipids, liver lipids, and used qPCR to quantify liver expression of PPARs alpha, beta, and gamma, as well as select downstream genes involved in PPAR-mediated signaling. Our data will provide fundamental understanding of the relationship between SMI and lipid stores in *X. laevis*, which may be an underutilized but effective fitness metric. Further, our data will test whether environmentally relevant PFAS exposures influence growth, development, body condition or lipid stores. Importantly, we will link any observed changes in lipid content or phenotypes to PPAR expression and signaling cascades, which may be an important mechanism of PFAS toxicity in vertebrates.

## 1.05 Enhanced Strategies and Best Practices for Identifying and Evaluating Endocrine System Adverse Effects

**1.05.T-01 Analysis of Historical Control Data for the Extended Amphibian Metamorphosis Assay**

***Katherine K. Coady**, Audrey Bone, Breanne Holmes, Laurent L.-M. Lagadic, Allen Olmsted and Lisa S. Ortego, Bayer CropScience LLC*

The extended Amphibian Metamorphosis Assay (eAMA), which is a modification of the amphibian metamorphosis assay test guideline (OCSPP 890.1100 and OECD 231), employs a fixed stage design terminating when *Xenopus leavis* tadpoles reach NF stage 62 rather than terminating at a fixed time after 21 days. Conducting an eAMA instead of a standard AMA avoids the confounding influence of different developmental stages on measurements of morphology and thyroid histopathology. In addition, the extension to NF stage 62 corresponds with metamorphic climax and an assessment of potential thyroid perturbations at a sensitive life-stage. Stage 62 also corresponds with the timing of thyroid-related metrics in the Larval

Amphibian Growth and Development Assay (LAGDA). After conducting ten eAMAs with a collective total of 14 control groups (*i.e.* n=10 negative controls and n=4 solvent controls), the measurement endpoints at test termination among the eAMA control groups were evaluated. This evaluation was conducted to characterize the range of responses and the associated variability among control organisms at stage 62. Overall, time to NF stage 62 was the most variable parameter with an overall coefficient of variation (CV) of 15.1%, while normalized hind limb length at stage 62 was the least variable parameter with an overall CV of 5.35%. Significant differences in growth and developmental timing were noted between the two labs that conducted the eAMAs. This was likely the result of different husbandry practices. In most incidences, solvent control responses were not significantly different compared to concurrent negative control responses (*i.e.* 75% of the comparisons), although a few statistical differences were noted in the data set. Characterizing the historical control database for the eAMA will be used to develop a decision logic for this modified test design and will also be used to understand the biological relevance of statistically identified differences among responses.

**1.05.T-02 Morphohistological Data of Normal Variation of the Thyroid Glands From Tadpoles in Amphibian Metamorphosis Assays Using Automated Image Analysis by Machined Learned Algorithms**

*Nils Warfving, Laura Polledo Ruiz, Alex Vitali, Bastian Zingg and* ***Klaus Weber****, AnaPath Services GmbH, Switzerland*

Knowledge of the morphological variation of normal thyroid glands in the corresponding developmental stage of tadpoles is a prerequisite for the assessment of possible induced endocrine effects by the toxicological pathologist. Heterogenicity among thyroid glands from control animals, may be associated with larval stage, breeder, facilities, alimentary conditions, possible solvent effects, etc. Histological sections of thyroid glands stained with haematoxylin and eosin from control tadpoles of 12 studies, n = 301 tadpoles stage [1] 57 to 60) from three test facilities (TF1, TF2, TF3) were evaluated by pathologists and the corresponding whole slide images were analysed using an automated procedure in QuPath (Bankhead P,et al 2017). In addition, the high dose group from a study with test-item induced hypertrophy was also analysed using the machine learned algorithms to evaluate the use of this tool to support traditional histologic evaluation. The data obtained include total thyroid gland area, ratio between the follicular epithelium vs. total area, height of the follicular epithelium, total colloid area, and ratio between epithelium and colloid area as well as number of epithelial cells.

The results provide a basis for understanding normal variation for specific larval stages, and data revealed differences in thyroid gland morphometry among test facilities. Furthermore, there were minor differences between controls in water and controls in solvents. Thus, thyroid findings need to be carefully interpreted and compared to study stage-matched controls for conclusive results.

The methodology was also applied to a study with induced thyroid gland hypertrophy and hyperplasia. Automated image analysis showed marked and significant differences between the control group and high dose for all parameters. It is concluded that image analysis by automated machined learned algorithms enabled objective quantitative data for the thyroid gland and across the complete 2D histological section. Therefore, the image analysis is considered a valuable and accurate tool to support the microscopic evaluation by the pathologist.

**1.05.T-03 Cytotoxicity Profiling to Help Differentiate Endocrine and Non-Endocrine Effects in the H295R Steroidogenesis Assay**

***Steven L. Levine****, Regulatory Science, Bayer CropScience LLC*

The adrenal gland is the most common toxicological target organ within the endocrine system. The adrenal gland is uniquely vulnerable to toxicity from contaminants because of its high perfusion rates and high lipid content. Understanding potential effects on adrenal endocrine function is an area that requires new and alternative toxicological methods. The OECD validated H295R steroidogenesis assay (test guideline 456) utilizes human adrenocortical carcinoma cells in culture to evaluate chemical effects on steroidogenesis (e.g., testosterone (T) and 17β-estradiol (E2) production). With steroidogenesis being a process where the rate limiting and commitment steps depend on mitochondrial enzymes, it is critical to assess cell viability and the state of the mitochondria to help differentiate between endocrine versus non-endocrine disruption of steroidogenesis. A battery of cytotoxicity assays has been used to inform on cell membrane integrity, mitochondrial function, and ATP quantification to comprehensively characterize chemical-elicited effects in H295R cells. Using a custom set of reference chemicals, this evaluation included 7 complementary assays to inform on potential modes of action for cytotoxicity and to help differentiate between endocrine and non-endocrine effects. For example, one can assess which chemicals cause effects on cell membrane integrity as opposed to mitochondrial effects such as depolarization or ATP production. Chemicals were evaluated at five test concentrations and after 48 hours of exposure, concentration-response effects on cell viability were assayed as well as quantifying T and E2 levels. For example, carbonyl cyanide m-chlorophenyl hydrazone (CCCP), a mitochondrial uncoupling agent, resulted in decreased T and E2 levels and elicited significant effects in the CellTiter-Glo, Mitotracker, and most notably the JC-10 assay which detects changes in mitochondrial membrane potential. These results confirm that CCCP impaired mitochondrial stability in H295R cells but did not cause overt cell death within the timeframe of the study. Thus, for CCCP mitochondrial disruption underlies the observed decrease in hormone production. Cumulatively, this study reveals that comprehensive assessment of cell and mitochondrial status is recommended to complement hormone data in order to differentiate specific versus non-specific cytotoxic effects on steroidogenesis.

**1.05.T-04 Influence of Systemic Copper Toxicity on Early Development and Metamorphosis in Xenopus laevis**

***Douglas J. Fort***[1]*, Kevin Todhunter*[1]*, Jeffrey C. Wolf*[2]*, Kevin Long*[3]*, Craig A. Poland*[3]*, Michael McGrath*[4]*, Stijn Baken*[5] *and Carol Mackie*[3]*, (1) Fort Environmental Laboratories, Inc., (2 )EPL, Inc., (3) Regulatory Compliance Limited, United Kingdom, (4) Necon Technologies Ltd, Ireland, (5) European Copper Institute, Belgium*

The purpose of the study was to examine if systemic toxicity induced by copper sulphate pentahydrate exposure during pre-metamorphic development in *Xenopus laevis* compromises early development, growth, and metamorphic processes independent of the hypothalomo-pituitary-thyroid axis (HPT) axis. A 28-day exposure study with copper sulphate pentahydrate, initiated at developmental stage 10, was performed using test concentrations of 3.0, 9.0, 27.2, 82.5 and 250 µg Cu/L. The primary endpoints included mortality, developmental stage (NF), embryo-larval malformation, behavioural effects, hindlimb length (HLL), growth (snout-vent length [SVL] and wet body weight), and histopathology. The 28-day LC50 value with 95% confidence intervals was 61.2 (51.4-72.9) µg Cu/L with 250 µg Cu/L resulting in complete lethality. Developmental arrest in the 82.5 and delay in the 27.2 µg Cu/L treatments was observed as early as study day (SD) 10 and continued throughout the remainder of exposure. SVL-normalized hind limb length (HLL), body weight, and SVL in the 27.2 and 82.5 µg Cu/L treatments were significantly decreased relative to control. At the concentration of 82.5 µg Cu/L, thyroid gland size was markedly reduced when compared to controls, which was consistent with the degree of developmental and growth arrest in those larvae. Concentration-dependent findings in the intestine, liver, gills, eyes, and pharyngeal mucosa were consistent with non-endocrine systemic toxicity. These were prevalent in the 9.0 and 27.2 µg Cu/L treatment groups but were minimally evident or absent in the 82.5 µg/L group. This lack of effects at the highest test concentration was attributed to developmental arrest. In conclusion, all marked developmental delay in frogs exposed at 27.2 and 82.5 µg Cu/L concentrations was the result of systemic toxicity that occurred early in development prior HPT-driven metamorphosis, and was not indicative of endocrine disruption.

**1.05.T-06 Site-Directed Mutagenesis of Human Type 3 Iodothyronine Deiodinase to Evaluate SeqAPASS Predictions of Cross-Species Susceptibility to Chemical Inhibition**

***Sally A. Mayasich***[1]*, Michael R. Goldsmith*[2]*, Kali Mattingly*[3] *and Carlie LaLone*[4]*, (1) University of Wisconsin, Madison, (2) Congruence TX, Chapel Hill, (3) SpecPro Professional Services Contractor to the U.S. EPA, (4)* *U.S. Environmental Protection Agency*

The potential for U.S. EPA's SeqAPASS (Sequence Alignment to Predict Across-Species Susceptibility) tool to predict susceptibility of type 3 iodothyronine deiodinase (DIO3) enzyme to chemical inhibition was investigated. The SeqAPASS tool identifies whether known critical amino acids involved in catalytic function are exact, partial, or not matches across species compared to a template species. Predictions of susceptibility are based on properties including molecular weight and side chain classification. From this evaluation, amino acid differences identified between vertebrates guided the selection of amino acids for site-directed mutagenesis (SDM) studies. Site-directed mutagenesis of the wildtype (WT) human DIO3 gene sequence was used to create six variant proteins expressed in cell culture which were tested *in vitro* for chemical inhibition. We found significant differences in *in vitro* $IC_{50}$ results, but generally within an order of magnitude, among variants for a set of chemicals selected as potential competitive inhibitors based on a previous screening of ToxCast chemicals. Three variants predicted by SeqAPASS as partial matches showed significant differences from WT $IC_{50}$ for 5 to 6 of the 9 chemicals screened, one amino acid change that was not a match in SeqAPASS resulted in differences from WT for 5 of the 9 chemicals, whereas another differed significantly for 8 chemicals. Another amino acid change that was not a match in SeqAPASS differed from WT for only one chemical. Hill slopes for the concentration-response curves did not differ significantly among variants for most chemicals, but steeper slopes for some chemicals indicated potential binding to more than one site. Virtual docking of the chemicals to structural models representing the WT and amino acid substitutions in other species support the hypothesis that certain chemicals are competitive inhibitors at the catalytic site and others may interact at a proposed cofactor site (interaction site of the reducing cofactor, dithiothreitol [DTT]). Differences in inhibition were only partially explained by side-chain properties of the substituted amino acid. Chemical characteristics and the location and putative function of the substituted amino acid in the hDIO3 enzyme are important considerations in understanding the potential for chemical susceptibility across species. *This abstract neither constitutes nor necessarily reflects USEPA policy.*

**1.05.T-05 Discussion - Enhanced Strategies and Best Practices for Identifying and Evaluating Endocrine System Adverse Effects**

***Ellen M. Mihaich***[1], *Jeffrey C. Wolf*[2] *and Scott G. Lynn*[3], *(1) ER2, (2) EPL, Inc., (3) U.S. Environmental Protection Agency*

**1.05.T-07 Faster and Easier!. . . but Better? "Key Characteristics" vs. Weight of Evidence for Identifying Endocrine Disruptors**

***Christopher Borgert***, *Applied Pharmacology & Toxicology, Inc.*

A new approach for identifying endocrine disrupting chemicals (EDCs) has been proposed that organizes data according to ten "key characteristics" of endocrine disruption, and evaluates chemicals according to data that imply those characteristics. This approach is based on the claimed success of 10 Key Characteristics for identifying carcinogens. Using scientific articles published in peer-reviewed journals through the end of 2020, we evaluated the Key Characteristics approach for EDCs conceptually and empirically for transparency, susceptibility to bias, and for consistency with principles of endocrine pharmacology and dose-dependence of mechanisms of action. We found deficiencies in the Key Characteristics approach for EDCs that are like those demonstrated previously for the ten Key Characteristics of carcinogens. The Key Characteristics approach for EDCs fails to apply the consensus definition of EDC and it is not amenable to empirical testing or validation. As formulated, it is intended to be flexible according to diverse goals, but this flexibility also ensures that it will produce inconsistent and unreliable results. The proposed Key Characteristics for EDCs ignores principles of hormone action and characteristics of dose-response in endocrine pharmacology and toxicology. The Key Characteristics approach for EDCs also lacks a means to reach a negative conclusion about a chemical's EDC properties and appears to be incapable of distinguishing EDCs from non-EDCs. The Key Characteristics approach for EDCs provides no means for developing a valid consensus among experts nor does it provide a means of resolving conflicting interpretations of data. A better approach to the identification of

EDCs would build upon validated Weight of Evidence approaches that incorporate critical components lacking in the proposed Key Characteristics approach for EDCs. Robust Weight of Evidence approaches organize data according to specific, testable hypotheses regarding potential endocrine modes of action, appropriately contextualize *in vitro* and *in vivo* assays and evaluate data quality and relevance for each specific, testable hypothesis. Weight of Evidence approaches also acknowledge the importance of mechanistic potency relative to endogenous hormones, the need to establish causality between mechanistic steps, and consider the dose-dependence of those mechanisms and their relevance in the context of human exposures.

**1.05.T-08 Key Characteristics Versus Weight of Evidence for Assessing Endocrine Disruption: Case Study on Glyphosate**

***Donna R. Farmer*** *and Steven L. Levine, Bayer CropScience LLC*

A weight of evidence (WoE) approach assesses all available information and weighs data by considering relevance, reliability, and quality. Using a WoE approach the US Environmental Protection Agency's Endocrine Screening Program in 2015, the European Food Safety Authority EFSA in 2017, a peer reviewed article by Bayer Crop Science in 2020, and the Assessment Group on Glyphosate, the rapporteur Member States who led the European Annex 1 renewal of glyphosate in 2021, all evaluated glyphosate 's potential to interact with the estrogen, androgen, thyroid and steroidogenic (EATS) pathways. These evaluations included results from *in silico* screening, the Endocrine Disruption Screening Program Tier 1 battery, guideline regulatory studies with endpoints that informed an endocrine assessment, and open literature studies. The reviews concluded that glyphosate does not have endocrine-disrupting properties through EATS modes of action. In striking contrast to these conclusions a recent paper, that evaluated glyphosate on the ten recently proposed key characteristics (KC) of endocrine-disrupting chemicals (EDCs), concluded that glyphosate satisfied at least eight KCs of an EDC. KCs are physicochemical properties commonly shared by EDCs that can elicit various mechanistic endpoints. Numerous relevant and reliable documents assessing glyphosate's potential endocrine activity and reproductive and developmental toxicity were not discussed. Rather, the authors focused on a small subset of publications that used non-standard *in vitro* / *in vivo* methods that tested glyphosate and its formulations often at unrealistically high concentrations exceeding those recommended for endocrine testing by international guidelines. Additionally, the studies were not designed to differentiate between endocrine and non-endocrine activity, which confounded the conclusions of the study. Furthermore, they did not critically evaluate the cited studies for relevance, reliability, and quality, which is required for systematic reviews. Also, they failed to use a WoE to determine whether the criteria for each key characteristic were met. In conclusion, application of KCs and not performing a WoE for evaluating glyphosate's potential to interact with the EATS modalities resulted in an incorrect classification.

**1.05.P Enhanced Strategies and Best Practices for Identifying and Evaluating Endocrine System Adverse Effects**

**1.05.P-Th009 Histological Lesions in African Clawed Frogs (Xenopus laevis) Used in the Amphibian Metamorphosis Assay**

*Olivia Richard,* ***Klaus Weber****, Laura Polledo Ruiz, Tanja Razinger and Bastian Zingg, AnaPath Services GmbH, Switzerland*

*Xenopus laevis* tadpoles are widely used in the Amphibian Metamorphosis Assay (AMA) to identify substances which may interfere with the normal function of the hypothalamic-pituitary-thyroid (HPT) axis. Although *Xenopus laevis* have been used extensively in research, there is a paucity of information in the current literature describing background lesions and their relevance. We retrospectively assessed data from 42 AMA studies from five institutions located in North America and Europe. To our knowledge, this is the first report documenting spontaneous background lesions in Xenopus laevis. This document serves as a resource to pathologists and will aid in interpretation of findings and differentiation of background from test article–related changes.

Forty-two studies have been used for this compilation. The in-life part was performed in four different European and one North American CRO's. All studies comprised a dilution (water) control group, whereas solvent controls where use for each acetone and TEG (triethylene glycol) in one study, and DMF (dimethylformamide) in 14 studies. In each study, there were 4 replicates per study and at least 20 tadpoles per group for histology evaluation. This resulted in the following number of control animals: water control – 843; DMF – 280; TEG – 25; acetone -20. From 23 studies, thyroid glands have been evaluated only. In 19 studies, also liver and/or kidney sections (transversal sections through body) were present, whereby all present organs have been screened in addition. The studies have been evaluated by three different pathologists from AnaPath Services GmbH.

No differences were found for thyroid gland alterations between control groups from different CRO's.

Background lesions of degenerative and/or inflammatory nature, as well as infections and parasites have been encountered in different organs: liver, kidneys, abdominal cavity, gills, gastrointestinal tract pancreas, skeletal muscle, skin, eyes, and oral cavity.

All inflammatory and degenerative background lesions may be encountered as induced findings, however, at higher severity/incidences. Up to now, renal tubular necrosis, renal granulomatosis inflammation, and metaplasia of gill epithelia have not been diagnosed in control animals. Different solvents did not cause different lesions. There were no major differences between different CRO's.

**1.05.P-Th010 Histological Lesions in Fathead Minnows (Pimephales promelas) Used in the Fish Short Term Reproduction Test**

***Klaus Weber***, *Paula Ortega Pérez, Laura Polledo Ruiz, Olivia Richard, Tanja Razinger and Bastian Zingg, AnaPath Services GmbH, Switzerland*

Fathead minnows are widely used in the Fish Short Term Reproduction Test (FSTR) to identify substances which may interfere with the normal function of the hypothalamic-pituitary-thyroid (HPT) axis.

The target organs for histopathological examination are the testes and ovaries. Liver and/or kidney are occasionally added per protocol. However, a number of background lesions can also be found in other organs, e.g., intestines, muscles, etc. They can be of inflammatory or degenerative nature, triggered by bacterial infections, parasites. Also, neoplasms are encountered. In addition, the test substances can induce not endocrine-related findings. Both, background findings and other induced findings must be considered to confirm, or neglect induced endocrine effects.

Therefore, we summarized retrospectively the findings of 50 studies evaluated at AnaPath Services GmbH by five pathologists. The in-life part of these studies was performed in four different European and one North American CRO's. All studies comprised a dilution (water) control group, whereas solvent controls (mainly DMF (dimethylformamide) but also acetone and TEG (triethylene glycol) where used in several studies. Different solvents did not cause different lesions. There were no major differences between different CRO's.

**1.05.P-Th011 Molluscs in Toxicology Testing Emphasizing Snails**

***Klaus Weber***, *AnaPath Services GmbH, Switzerland*

The extent of possible effects of chemicals on mollusks came into public regard with the dramatic effects of tributyltin (TBT) compounds, which have broadly been used as antifouling agents for ships. The females of the Dog Whelk (*Nucella lapillus*) and of at least 160 further species exposed to TBT developed male parts in addition to the female genital organs, a syndrome named 'imposex'.

Therefore, a review paper of the OECD (2010) recommended test procedures for optimization and possible validation for partial life cycle (PLC) test *P. antipodarum* (freshwater, gastropod), full life cycle (FLC) with *L. stagnalis* (freshwater, gastropod), and *Crassostrea gigas* (bivalve, marine). It was also recommended to develop protocols for FLC with *P. antipodarum* and PLC with *L. stagnalis*. The OECD (2016 a, b) published a guideline for a reproduction test with *L. stagnalis* and *P. antipodarum*. Histopathology was detailed in the guidelines but stated as '…other endpoint (e.g., histopathology)…' (OECD, 2016b) and was mentioned under point 3.3. and in the Annex for possible histopathology evaluation (OECD, 2016a).

The current guidelines target mainly endocrine disruptor effects. However, inflammatory, and degenerative processes or parasitic infestations can mask or mimic endocrine effects. Therefore, a histopathological examination should be performed. The cost and labor are not high.

**1.05.P-Th012 Comparison of Xenopus laevis NF developmental stage-matched control data in Amphibian Metamorphosis Assay continuous quantitative endpoints (HLL, SVL, wet weight)**
*Joseph Marini[1], Lee Sayers[1], Maria Estebaranz-Yubero[2], Apostolos Koutsaftis[2], Oliver Körner[3], Sonja Haaf[3] and* ***Douglas J. Fort****[4], (1) Smithers, (2) ERM Regulatory Services Limited, United Kingdom, (3) ADAMA Deutschland GmbH, Germany, (4) Fort Environmental Laboratories, Inc.*
The analysis of continuous quantitative Xenopus laevis endpoints such as snout-vent length (SVL), hind limb length (HLL), nHLL (normalized by SVL) and wet weight is performed to assess statistically significant differences between the negative control and active substance treatments in the Amphibian Metamorphosis Assay (AMA) for the detection of potential thyroid modes of action according to Guidelines OECD 231 and OPPTS 890.1100. However, the current statistical analysis of these parameters at Day 7 and 21 is routinely performed by using the replicate means or medians of the recorded HLL, nHLL, SVL, and wet weight individual values without taking into consideration the different stages of development within each control or treatment replicate. Furthermore, these endpoints are well known to be directly impacted by developmental stage in tadpoles and so using stage-matched data may have an impact in the interpretation of the biological results. This is also the case for tadpole thyroid gland histopathological findings, where it is already recognized in both Guidelines that the most appropriate approach is to use NF developmental stage-matched tadpoles for evaluation. Consequently, individual control HLL, nHLL, SVL, and wet weight values for Day 7 and 21 from the available data set have been extracted and evaluated according to NF developmental stage. The analysis presented here aims to develop a NF developmental stage-matched Historical Control Database (HCD) that allows the assessment of potential impact in the outcome of statistically significant results of these thyroid screening parameters, as well as the variability of NF developmental stage-matched control data across different GLP AMA-performing laboratories.

**1.05.P-Th014 Adverse thyroid and neurodevelopmental effects of DiNP in GH3 cell and zebrafish (Danio rerio): A comparison with DEHP**
***Yunchul Ihn****, Yoojin Cho, Inae Lee and Kyungho Choi, Seoul National University, Korea, Republic of (South)*
Diisononyl phthalate (DiNP) has been used in increasing amount to replace bis(2-ethylhexyl) phthalate (DEHP), and hence been widely detected in environment and humans. While DiNP is suggested for its anti-androgenic effects, less is known for its thyroid-disrupting and neurodevelopmental potentials. Adverse thyroid-disrupting potential of DiNP and/or its major metabolites were assessed by gene transcription or thyroid hormone levels using a rat pituitary carcinoma cell line (GH3) or embryolarval zebrafish (*Danio rerio*), and compared with those observed by DEHP exposure. In the larval fish, neurobehavioral changes along with transcription of neurodevelopmental-related gene were also evaluated. DiNP exposure caused significant increases in thyroid hormone and decreased behavior in the larval fish, which were similar to those observed from DEHP. In GH3 cell, however, exposure to DiNP metabolites showed gene alteration profiles which were different from those of DEHP. In larval zebrafish (5 day post fertilization), dose-dependent increase in thyroid stimulating hormone (TSH), total thyroxine (TT4), total triiodothyronine (TT3), and free triiodothyronine (fT3) levels were seen after

DiNP exposure (0, 1.0, 3.0, 6.0 mg/L), with up-regulation of genes related to central regulations (*crhβ*, *trh*, *tshβ*), synthesis (*nis*, *tg*, *tpo*, *hhex*, *nkx2.1a*), and metabolism of thyroid hormones (*dio1*, *dio2*, *dio3a*, *ugt1ab*, *sult1st5*). In GH3 cell, DiNP, mono-isononyl phthalate (MiNP), and mono-carboxy-isooctyl phthalate (MCiOP) up-regulated *tshβ* and *dio2* expression which were comparable to the changes seen *in vivo*, while mono-hydroxy-isononyl phthalate (MHiNP) showed the opposite direction of transcriptions, suggesting that DiNP and its metabolites might show different endocrine disrupting potentials. Also, DiNP (0, 0.3, 1.0, 3.0, 6.0 mg/L) and DEHP (0, 0.03, 0.1, 0.3, 1.0 mg/L) exposure in larval zebrafish decreased total distance moved and mean velocity of the fish in light and dark stimuli. Down-regulation of neurodevelopmental genes (*c-fos*, *elavl3*, *ngn1*, *nestin*, *mbp*) were also detected. As thyroid hormones are considered one of important mechanisms that may lead to developmental neurotoxicity in early life stages, the changes observed in this study suggest that DiNP may affect neurobehavior of the fish through thyroid alteration. Overall, these results emphasize the need for consideration of adverse thyroid and neurodevelopmental effects in regulations of phthalates replacing DEHP.

**1.05.P-Th015 Effects of reproductive endocrine system in male zebrafish exposed to mixtures of avobenzone and homosalate**

***Sujin Lee***[1] *and Kyunghee Ji*[2]*, (1) Korea, Republic of (South), (2) Yongin University, Korea, Republic of (South)*

Avobenzone (AVB) and homosalate (HS) are often used as ingredients in sunscreens that absorb sunlight and prevent UV penetration into the skin. Although the individual toxicity of these substances is often reported, there is limited information on the mixture toxicity. In this study, the effect on the reproductive endocrine system was observed after exposure to male zebrafish for 21 days by mixing AVB and HS. Changes in organ weight compared to body weight, sex hormone levels, and changes in transcription of genes related to hypothalamus-pituitary-gonad axis were investigated. At the histological level, the gonadosomatic index of zebrafish exposed to HS and the mixture was significantly decreased while the hepatosomatic index was significantly increased in both groups. Testosterone levels in male zebrafish exposed to HS and mixture were significantly decreased, and this effect was greater in the mixture group. These results suggest that HS induces an anti-androgen effect in males, and this effect may be greater when co-exposed with AVB. The potential consequences of toxic effects considering the ratio of UV-filter substances in actual products deserve further investigation. Acknowledgement: This study was supported by National Research Foundation of Korea (NRF; Project no. 2019R1A2C1002712).

**1.05.P-Th016 Toxic effects of four UV-filters on the thyroid endocrine system and development in zebrafish**

***Yujin Ka*** *and Kyunghee Ji, Yongin University, Korea, Republic of (South)*

Avobenzone (AVB), octinoxate (OMC), homosalate (HS), and octisalate (OS) are widely used as organic UV-filters and have been frequently detected in aquatic environment. Although their estrogenic or anti-androgenic properties have been investigated, limited information is available on thyroid endocrine disruption. In this study, we evaluated the potential of thyroid endocrine disruption of AVB, OMC, HS, and OS using wild-type and dre-miR-499$^{-/-}$ zebrafish. To better understand the underlying mechanism, concentrations of triiodothyronine (T3) and thyroxine (T4) hormones and transcription of genes involved in the hypothalamus-pituitary-thyroid axis were measured. Toxicity of HS and OS was greater than that of AVB and OMC considering the endpoints of hatching, survival, and growth. Significant increase of T3 concentrations, up-regulation of *trα* and *trβ* genes, and down-regulation of *crh* gene were observed in fish exposed to HS and OS. Significantly reduced survival in dre-miR-499$^{-/-}$ zebrafish exposed to HS and OS suggests that this microRNA plays an important role in the toxicity of both chemicals. Potential developmental effects and thyroid endocrine disruption by long-term exposure to HS and OS deserves further investigation. Acknowledgement: This study was supported by National Research Foundation of Korea (NRF; Project no. 2019R1A2C1002712).

**1.05.P-Th017 Characterizing Neuroendocrine and Neurotoxic Effects of Bifenthrin to Salmonids and Influence of Climate Change to Toxicity: An Integration of Omic Profiles to Apical Endpoints**

***Jason Tyler Magnuson***[1] *and Daniel Schlenk*[2], *(1) University of Stavanger, Norway, (2) University of California, Riverside*
An increase in the application of pyrethroid insecticides in the San Francisco Bay Estuary and Sacramento San Joaquin Delta has raised concern for the populations of several salmonid species. Bifenthrin (BF), a type I pyrethroid, is among the most frequently detected pyrethroids in the Bay-Delta watershed. Predicted climate change effects, such as increasing water temperature in fish rearing habitats, is an additional stressor to salmonids in the Delta. To better characterize the effects of BF and influence of changing environmental conditions, salmonids were exposed to concentrations of BF detected in the Delta (30 ng/L-1.5 µg/L) under various water temperatures (11, 16.4, and 19 °C) as predicted by recent climate change models. When co-exposed to BF under increased water temperature scenarios, there was a heightened decrease in dopamine levels and dysregulation of genes involved in dopaminergic and neuroendocrine processes in the brains of Chinook salmon and steelhead trout with increasing temperature. These changes were further reflected when Chinook were assessed for olfactory function, with BF exposed fish less deterred from predatory odorant cues, relative to controls. To better understand the underlying effects of BF to neuroendocrine function, non-targeted metabolomic studies were conducted on Chinook and steelhead following BF treatments. Alterations in the levels of several metabolites in the brains of exposed fish were predicted to induce an apoptotic, inflammatory, and reactive oxygen species response. Transcriptomic analysis revealed that BF exposed trout had predicted pathways involved in gonadotropin releasing hormone signaling, reduced extracellular matrix stability and adhesion, as well as cell death. Histopathological analysis subsequently showed an increased number of TUNEL positive, apoptotic cells in the brains of BF exposed fish. Non-targeted metabolomic and transcriptomic profile analyses were integrated to better characterize a common target of BF in the brains of exposed salmonids. Pathways involved in the metabolism of triglycerides were predicted to be a novel target of BF in the brains of exposed fish. Lipidomic analysis was further conducted and a dose dependent decrease in the abundance of triglycerides was observed by BF. Overlaying multi-omic level molecular responses and subsequently linking them to histopathological and behavioral effects has allowed us to find specific targets of BF in the brains of exposed salmonids.

**1.05.P-Th018 Thyroid Endocrine Disruptor Screening System Using Thrαa-/- and Dre-miR-499-/- Knock-out Zebrafish**

***Inhye Lee***[1], *Suhyun Park*[2] *and Kyunghee Ji*[1], *(1) Yongin University, Korea, Republic of (South), (2) Seoul National University, Korea, Republic of (South)*
Environmental contaminants that affect the thyroid endocrine system can eventually cause developmental retardation or metabolic problems. A number of *in vitro* cell-based assays have been developed to screen potential thyroid endocrine disrupting chemicals (EDCs). However, these assays are not suitable for identifying changes *in vivo* system.  The purpose of the present study was to establish methods to screen for thyroid EDCs at high throughput and to precisely characterize the underlying mechanism in *in vivo* system. We analyzed the survival of two types of knockout zebrafish larvae (thyroid hormone receptor alpha a (thrαa)$^{-/-}$ and dre-miR-499$^{-/-}$) to T3, propylthiouracil, and methimazole. We confirmed that in the new assay, monitoring survival rate for only 4 days is sufficient to detect thyroid endocrine disruption. We also intended to test the utility of this novel method as a tool to precisely characterize the thyroid endocrine disruption mechanism of given chemicals. To this end, we selected bisphenol A and its alternatives, di-2-ethylhexyl phthalate and its alternatives, and isothiazolinones as  model chemicals. We demonstrated that our new protocol indeed works and knockout zebrafish embryos provide a means for rapid and specific screening of thyroid endocrine disruption.
Acknowledgment: This study was supported by the National Research Foundation of Korea (NRF; Project no. 2019R1A2C1002712).

**1.05.P-Th020 An In Silico Approach for Biomarker Discovery of PFAS Toxicity Effects in Fish**

***Rayna M. Nolen***, *Antonietta Quigg and David Hala, Texas A&M University at Galveston*
Per and poly-fluoroalkyl substances (PFAS) are anthropogenic ‘emerging’ pollutants that are used in various

domestic and commercial applications (such as cookware, food packaging, etc.). PFAS pollutants have been detected in the surface waters and fish tissues from Galveston Bay, TX. Therefore, there is an urgent need to assess whether PFAS exposure could cause toxicity effects in aquatic wildlife. In this presentation, I describe the results of my research using an *in silico* computational biology method to identify the likely biomarkers of PFAS toxicity. Already available RNA-sequencing datasets from zebrafish toxicity studies with PFAS (from the NCBI's GEO website) were parsed for transcriptomics changes of metabolic enzyme genes. An *in silico* stoichiometric model of zebrafish metabolism was used to integrate transcriptomics changes for metabolic enzymes as constraints onto the zebrafish model. Subsequently, flux balance analysis (FBA) and its extensions were used to simulate the effects of exposure on candidate metabolites and reaction sub-systems that may serve as diagnostic biomarkers of toxicity or stress effects. Simulations indicate dyslipidemia (fatty acid metabolism), which agrees with the findings from *in vivo* toxicological studies.

**1.05.P-Th021 Development and assessment of Adverse Outcome Pathways for juvenile hormone mediated effects of environmental stimuli and chemicals in cladoceran**

***Haruna Watanabe***[1]*, Kenji Toyota*[2]*, Masashi Hirano*[3]*, Ryoko Abe*[1]*, Hitoshi Miyakawa*[4]*, You Song*[5]*, Knut Erik Tollefsen*[5,6,7]*, Hiroshi Yamamoto*[1] *and Taisen Iguchi*[8]*, (1) National Institute for Environmental Studies, Japan, (2) Niigata University, Japan, (3 )Tokai University, Japan, (4) Utsunomiya University, Japan, (5) Norwegian Institute for Water Research (NIVA), (6) Norwegian University of Life Sciences (NMBU), (7) Centre for Environmental Radioactivity (CERAD), Japan, (8) Yokohama City University, Japan*

Juvenile hormone (JH) is a multifunctional hormone regulating larval development, molting, metamorphosis, reproduction, and phenotypic plasticity in arthropods. Many JH agonists have been designed to control harmful insects in agriculture and aquaculture and their adverse effects such as metamorphosis defects and larval lethality on nontarget species by disrupting JH signaling pathway are also concerned. Among wide Cladocera genera, which produce by parthenogenesis under appropriate environmental condition, JHs and JHAs have been demonstrated to induce dose-dependent increase in male offspring and reproduction decrease. In addition, environmental stimuli such as short-day condition also induce male offspring; thus, mechanistic understanding of the JH-mediated effects of both JHA and environmental stimuli in cladoceran based on the current knowledge is important. In this study, we constructed three linear Adverse Outcome Pathways (AOPs), which consist of the JH synthesis pathway leading to male offspring induction (AOP1) and the JH receptor (JHR) mediated pathway initiated by JHR agonists leading to male offspring (AOP2) and reproduction decline (AOP3), which finally results in population decline. The weight of evidence (WoE) evaluation of the proposed AOPs were performed to assess the essentiality of the key events (KEs) and the biological plausibility, empirical support, and quantitative understanding of each KE relationship. Short-day condition and several chemicals are considered to disrupt JH synthesis pathway and consequently increase JH titer. Both increased endogenous JH and JHA activate JH receptor (JHR) which increase expression of sex differentiation gene resulting in male offspring production. Biological and chemical domain of the AOPs and regulatory application the AOPs were also investigated to suggest potential use of detection assays for upstream KEs such as in silico molecular docking simulation of chemicals with JHR and in vitro reporter gene assay as high-throughput screening assays. After prioritization of potential JH disruptors by these assays, in vivo short-term JH activity screening assay (JHASA), which observe offspring sex produced after exposure of chemicals to adult female can be used to detect male offspring production. Based on the AOP evaluation, we could suggest the future direction of development of new assessment approach to complement current test methods and research needs to support the AOPs.

**1.05.P-Th022 Investigating the cause of Largemouth Bass intersex in an urban wastewater dominated river**

***Victoria McGruer***[1]*, William Ota*[1]*, Kali Krishnan*[1]*, Brian Yacoub*[1]*, Charles S. Wong*[2]*, Wenjian Lao*[2]*, Kurt E. Anderson*[1] *and Daniel Schlenk*[1]*, (1) University of California, Riverside, (2) Southern California Coastal Water Research Project (SCCWRP)*

The Santa Ana River flows within the largest river basin in southern California, home to over 5 million residents. Due to upstream dams and dry climate, the surface flow is dominated by wastewater effluent most of the year. The continuous input of treated effluent raises concern for protecting native fish within the river, such as the federally threatened Santa Ana sucker (*Catostomus santaanae*). A 2019 survey of Largemouth bass (*Micropterus salmoides*) found that the percent of fish with the visible presence of both testes and ovaries increased with proximity to a wastewater treatment plant (WWTP) outflow (Adjusted R-squared: 0.854, p-value: 0.015). In this study, we investigate the persistence of intersex within Largemouth bass in the Santa Ana River and the presence of estrogenically active compounds in several environmental matrices which may contribute to the intersex phenotype. In September 2021, water, sediment, periphyton, macroinvertebrates, and Largemouth bass were sampled at six sites along the Santa Ana River throughout Riverside, CA. Three sites were directly downstream of a WWTP. Two sites were further downstream from WWTP input (>1500 m). One site was a tributary to the Santa Ana and separate from any wastewater input. Additionally, water samples were taken directly from three distinct WWTP outflows. Histological analysis of male Largemouth bass testes found that some males at all sites displayed the presence of ova-testes ranging from 33-67% presence depending on the site. However, Intersex prevalence was not statistically different between sites (Chi-square test, $p = 0.7$), suggesting that endocrine disruption may be occurring at all sites. Chemical analysis found that the estrogens 17β-estradiol (E2) and estrone (E1) were detected at three and four of the six river sites, respectively. E1 concentrations in the river samples ranged from 0.27-1.7 ng/L, but concentrations at the WWTP outflows were below the detection limit. E2 river concentrations ranged from 0.28- 0.93 ng/L and E2 was detected at one WWTP at a concentration of 0.77 ng/L. Ethynyl estradiol was not detected at any sites sampled (< 0.222 ng/L). Water samples are currently being assessed using *in vitro* cell bioassays to determine if compounds other than E1 and E2 demonstrate estrogen receptor activity. Largemouth bass livers will be assessed for vitellogenin mRNA expression, a biomarker of exposure to estrogenic compounds.

**1.05.P-Th023 Sex Hormone Disruption Potentials of Major Polyhalogenated Carbazoles (PHCZs) in Human Adrenocortical Carcinoma (H295R) Cell Line**

***Yeonju Nam****, Ba Reum Kwon, Ah-Reum Jo, Yunchul Ihn, Eun-Jin Kim, Yoojin Cho, Gowoon Lee, Inae Lee and Kyungho Choi, Seoul National University, Korea, Republic of (South)*

Polyhalogenated carbazoles (PHCZs) are a group of emerging persistent organic pollutants (POPs) which are frequently found in the environment. Due to their structural similarity to dioxins, their toxicological effects are of growing concern but studies are mostly limited on development and cardiotoxicities. To fill the knowledge gaps on sex hormone disruption, the present study was designed to understand sex hormone disruption potentials of major PHCZs focusing on steroidogenesis pathways using human adrenocortical carinoma (H295R) cell line. Seven PHCZs of 2-BCZ, 2-CCZ, 3-BCZ, 2,7-BCZ, 3,6-BCZ, 3,6-CCZ, and 1,3,6,8-BCZ were chosen and noncytotoxic doses were applied to H295R cells for 48 hours. At the end of exposure, the cells were harvested to evaluate for sex hormone levels along with transcriptional changes related to steroidogenic genes. Following exposure, significant decrease of testosterone (T) were observed for all tested PHCZs. Up-regulation of *CYP19a1* gene by exposure to all seven PHCZs support the decreased T concentrations. Moreover, exposure to 3,6-BCZ significantly increased estradiol (E2) /T ratio. The results of this study demonstrate sex hormone disrupting potentials of PHCZs in H295R cells. Further animal studies are warranted to validate the estrogenicity observed for these chemicals.

**1.05.P-Th024 Thyroid Hormone Disrupting Potential of Acetyl Tributyl Citrate (ATBC) in embryo-larval Zebrafish (Danio Rerio)**

***Yoojin Cho****, Yunchul Ihn, Inae Lee and Kyungho Choi, Seoul National University, Korea, Republic of (South)*

Acetyl tributyl citrate (ATBC) is a citrate that has been used as plasticizer to replace DEHP. ATBC is used for a variety of purposes including food additives, product coatings, and pharmaceutical excipients, and hence been detected in indoor environment and products including processed foods, packaging, and medical tubing. Because of a lack of information on major metabolites of ATBC, biomonitoring studies, and consequently epidemiological reports on ATBC have not been conducted. In the present study, we screened thyroid hormone disruption potential of this important di(2-ethylhexyl)phthalate (DEHP) substitute, using zebrafish embryo. Changes in major gene transcription related to thyroid hormone homeostasis, and thyroid hormones were analyzed in whole body embryo-larval zebrafish after 5 d exposure. Embryos were exposed in 1 L beakers to a series of concentrations: SC (DMSO 0.01% v/v), 0.03, 0.1, and 0.3 mg/L. For each concentration, four replicates of 300 embryos were employed. At 5 days post fertilization (dpf), dose-dependent increase in total thyroxine (TT4) levels and decreased in total triiodothyronine (TT3) levels were observed in zebrafish larvae. Several genes of HPT axis showed alterations of transcription. *tshβ* and *crhβ* genes associated with the central regulations were upregulated. In addition, *tg*, *tpo* genes responsible for thyroid hormone synthesis, supporting increased TH levels in whole body larvae. Genes such as *ttr* and *sult1st5* which are related to thyroid hormone transportation and elimination were also upregulated. These results suggest that ATBC has a potential to disrupt thyroid hormone balance in the early life stage zebrafish, which are comparable to reported observations for DEHP. This is the first observation to show potential thyroid disrupting effects of ATBC in zebrafish. Further studies on its consequences in later life stage are warranted.

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### 1.05.P-Th025 Differential Isoform Usage in Fathead Minnows After Estrogenic Exposure

***Janine Fetke**, John Martinson and Adam Biales, U.S. Environmental Protection Agency*

The ability to produce different transcripts from the same gene through alternative splicing, known as isoform switching, is important for dynamic regulation of gene expression. Differential isoform usage allows for greater functional diversity of the genome, and aberrant isoform switching is associated with disease such as cancer. Gene expression response to estrogen exposure have been thoroughly described in fish, however little is known about isoform expression profiles for genes responsive to estrogen exposure. This study examines isoform usage in brain and liver tissues from 8-10 month-old male fathead minnows (*Pimephales promelas*) after a 48h exposure to either nominal 2.5 ng/L or 10 ng/L of 17α-ethynylestradiol (EE2) as well as untreated female fathead minnows. We used RNAseq data generated from brain and liver tissues and the software package, RSEM, for quantification of isoform abundance for the purpose of characterizing isoform usage across tissues, sex, and treatment. The identification of alternative splicing events indicates differential isoform usage as a potential mediator of organismal response to chemical exposure.

## 1.05.V Enhanced Strategies and Best Practices for Identifying and Evaluating Endocrine System Adverse Effects

### 1.05.V-01 Synergistic Activation of Estrogen Receptor Alpha by Two Biocides with Different Substances in Consumer Chemical Products in HeLa9903 Cells

***Sunmi Kim**[1], Daeyoung Jung[2], Hyun Young Kim[2], Kyounghee Kang[2], Woo Kyu Park[2], Heeyeong Cho[2] and Jongwoon Kim[2], (1) Seoul National University, Korea, Republic of (South), (2) Korea Research Institute of Chemical Technology (KRICT), Korea, Republic of (South)*

Many of the substances widely used in consumer chemical products are known as estrogenic endocrine-disrupting chemicals. Further, when they are exposed to receptors together, the interaction, i.e., antagonism, additivity, and synergism, between chemicals should be considered, but relevant experimental data are still lacking. For this reason, high-throughput screening of 352 chemicals and their binary mixtures were tested using HeLa9903 with miniaturing modification of OECD TG 455. As a result, we found that two biocides act as synergists. Most combinations with those two biocidal substances show over 2-fold more substantial estrogenic effects than expected. After combined and individual exposures, we further characterized the transcriptome and metabolome to determine the biological reason for synergism. Suppose these two chemicals co-expose in addition to the baseline effect of chemicals in our daily life; more severe estrogenic activity can perturb the homeostasis of the endocrine system. Validation studies in *in vivo* and epidemiological observations should be warranted.

**1.05.V-02 Deep learning model for synergistic effect prediction of estrogen receptor agonist for binary mixture**

***Myungwon Seo***[1]*, Daeyoung Jung*[1]*, Hyun Young Kim*[1]*, Woo Kyu Park*[1]*, Heeyoung Cho*[1]*, Jiwon Choi*[1]*, Sunmi Kim*[2] *and Jongwoon Kim*[1]*, (1) Korea Research Institute of Chemical Technology (KRICT), Korea, Republic of (South), (2) Seoul National University, Korea, Republic of (South)*

Endocrine-disrupting compounds (EDCs) manufactured in the global chemical industry are causing potential health threats. Among them, estrogenic EDCs interact with estrogen receptors (ER), affect many functions such as reproduction and metabolism, and induce mixture toxicity as various combinations of EDCs. In order to screen the mixture toxicity of estrogenic EDCs, experimental toxicity tests cannot perform on every possible mixture because of enormous mixture combinations. Non-testing methods, such as quantitative structure-activity relationships (QSARs) and deep learning, have been developed to predict the potential estrogenic activity of EDCs and overcome the limitation of toxicity tests. In a previous study, QSAR models for ER activity prediction based on a single chemical were developed by the collaborative estrogen receptor activity prediction project (CERAPP) and applied to software tools such as Danish QSAR and OECD QSAR Toolbox. However, since QSAR models were developed based on a single chemical, it is not suitable for predicting the synergistic effect of ER agonists in binary mixtures. In this study, we developed a deep learning model to predict the synergistic effect of ER agonists in binary mixtures. We constructed a high throughput screening system (HTS) based on the OECD test guidance 455 for predicting the synergistic effect of ER agonists and produced a dataset of ER agonist activity in a binary mixture. We classified labels into potential synergistic (positive value) and non-synergistic (negative value) effects based on the model deviation ratio (MDR) between observed and predicted values by the additive toxicity model. Mixture descriptors of the binary mixtures were calculated as composite properties based on the mole fraction of the mixture and physicochemical properties and biological data such as protein, gene, metabolic pathway, disease, etc. We performed data-balancing using the over- and under-sampling methods and classified datasets into internal and external validation datasets. The prediction model was evaluated using the external validation datasets. The model's performance was evaluated as accuracy, the area under the curve (AUC), sensitivity, and specificity. The deep learning model showed the potential to predict the synergistic effect of binary mixtures of ER agonists.

**1.05.V-03 New Japanese Program on Endocrine Disruption Succeeding EXTEND2016**

***Kunihiko Yamazaki****, Ministry of the Environment, Japan*

Ministry of the Environment, Japan (MOEJ) has been conducting testing and assessment of endocrine disrupting effects of chemicals under the program "EXTEND2016" (Extended Tasks on Endocrine Disruption) since June 2016. Two-tiered framework for testing and assessment of endocrine disrupting effects of substances was established and test methods were developed under international cooperation, contributing to establishment of international standard test protocols using fish, amphibians, and invertebrates. For substances selected referring to their potential environmental exposure in Japan, existing literature was collected and evaluated to identify candidate chemicals for testing, and relevant *in vitro* and *in vivo* tests have been conducted.

Based on internal review of achievements of EXTEND2016, MOEJ is preparing its succeeding program on endocrine disruption. It will maintain the structure of the preceding one, including risk-based approach to endocrine disrupting effects using definitive tests listed in the OECD Conceptual Framework for Endocrine Disruptor Testing and Assessment (EDTA), and will include some new elements. Based on the two-tiered framework already adopted, test protocols under development should be finalized and testing and assessment should be conducted to identify substances with significant environmental risk. In addition to *in vivo* and *in* *vitro* testing, New Approach Methodologies (NAMs) will also be considered to improve effectiveness and efficiency. To encourage appropriate risk management, development of new procedures on testing and assessment for respective existing regulatory frameworks will be attempted.

The succeeding program is to be finalized and published in the third quarter of 2022. Its objectives and outlines, as well as achievements of EXTEND2016, will be presented at the Annual Meeting.

**1.05.V-04 Influence of Storage Method and Duration on Plasma Vitellogenin Concentrations for Fathead Minnows**

***Julie Krzykwa****, Joseph Marini and Lee Sayers, Smithers*

Plasma vitellogenin (VTG) concentration is one of the primary apical endpoints of regulatory test methods using fish models where estrogen/androgen/steroidogenesis modalities are evaluated (e.g. OECD 229/230/234 and OCSPP 890.1350). With current European Union regulations mandating that all biocides and plant protection products be evaluated for potential endocrine disrupting effects—and the prevalence of VTG as an apical endpoint in such studies—there has recently been concern regarding the high intra- and inter-laboratory variation in reported plasma VTG concentrations and the potential impacts of this variation on the robustness of associated assays. One potential source of variation is sample storage. It is currently unclear how storage methodology and storage duration influences the stability and subsequent analysis of fathead minnow plasma VTG. While relevant guidelines outline a storage procedure for plasma samples (i.e., store with a protease inhibitor at -80 °C), it is clear from the literature that not all laboratories are using the described procedure. In addition, these guidelines provide no clear indication of the stability of samples under this storage regime. The paucity of information regarding the effects of storage method and duration makes it difficult to determine if these factors may be influencing the previously noted variation in VTG concentrations. Therefore, the objective of this project was to evaluate the impacts of storage method and storage duration on plasma vitellogenin concentrations. To achieve this objective, composite plasma samples from male and female fathead minnows were stored under multiple storage scenarios (i.e., with/without a protease inhibitor at -20°C or -80°C) and sample VTG concentrations were measured via ELISA at time points over an extended period. The results of this project will provide valuable information regarding the influence of storage method and duration on measured plasma VTG concentrations in fathead minnows.

**1.06 Healthy Water - Indigenous Community-Led Water Management Through Traditional Ecological Knowledge (TEK)**

**1.06.T-01 Introductory Remarks - Healthy Water - Indigenous Community Led Water Management Through Traditional Ecological Knowledge (TEK)**

***Stacey Fernandes****[1], Mandy Olsgard[2], James M. Lazorchak[3] and Brenda Rashleigh[3], (1) Canada North Environmental Services (CanNorth), (2) Integrated Toxicology Solutions Ltd., Canada, (3) U.S. Environmental Protection Agency*

**1.06.T-02 Integration of TEK into Exposure-Based Release Limits for Effluents**

***Stacey Fernandes****, Katherine Woolhouse and Caroline Lucas, Canada North Environmental Services (CanNorth), Canada*

There can be a challenge in integrating “two-eyed seeing”, which respects Indigenous knowledge and Western science, into the management of effluents. One method is to develop effluent release limits where the selected levels of protection in the receiving environment are set in discussion with the local Indigenous users.

Effluent releases to water can affect water quality, sediment quality, aquatic biota (e.g. fish, invertebrates), semi-aquatic wildlife (e.g. ducks, muskrat) and/or human health. Predictive models that consider contaminant movement through a watershed and the food chain can be useful tools for setting Exposure Based Release Limits (EBRLs) by allowing the back-calculation of the effluent release load based on meeting selected concentrations in various environmental components.

TEK can be integrated into the overall description of the watershed (flow changes, biology). The level of protection in the environment that are used to derive EBRLs can be set in discussion with local Indigenous users. There may be different levels depending on the location, for example meeting water quality guidelines may be appropriate in some locations, while at culturally important locations, more restrictive levels could be selected.

Examples of EBRLs derived for a hypothetical facility will be provided. Once derived, the EBRLs can then be used by the facility operator for planning purposes in the determination of Effluent Quality Criteria which would also consider other factors such as regulatory limits, technology-based release limits, pollution prevention, and cost-benefit analysis, while respecting TEK and protection of the environment.

**1.06.T-03 Incorporating Traditional Ecological Knowledge into the Risk Assessment and Cleanup Process at Contaminated Sites**

***Michelle Krasnec*** *and Kaylene Ritter, Abt Associates*

Indigenous communities are at greater risk of exposure to contamination and associated health risks compared to the general population due to their intrinsic relationships with the land, subsistence diets, and the fixed boundaries of reservations, which limits the ability to distance from contamination. To protect Indigenous communities from health risks due to contamination on their lands, meaningful engagement between the affected Indigenous communities and the federal agencies involved in cleanup and remediation efforts is essential. This process involves working with the affected Indigenous communities early and often to accurately characterize traditional practices that may lead to risk to community members, and to inform any potential remedial actions. Indigenous communities have the right to follow traditional practices in a manner that does not infringe on their health, and correctly characterizing health risks will help achieve this goal by improving the identification of appropriate cleanup actions.

Many Tribes have established contaminant cleanup standards and federally-approved water quality standards which are effective under the Clean Water Act. Often these Tribal standards are based on fish, gathered plant, and drinking water consumption rates corresponding to traditional diet and cultural practices specific to the Tribe. These standards should be considered during the risk assessment process. If a Tribe does not have promulgated standards, traditional ecological knowledge should still be considered in the risk assessment process.

Here we present multiple case studies demonstrating the importance of incorporating traditional practices to accurately characterizing health risks to Indigenous communities. We present case studies focused on contaminated surface water and sediments that impact contaminant concentrations in fish and gathered aquatic plants that may be consumed by the community. We will demonstrate the importance of characterizing the amount of fish consumed, the types of fish consumed, and the way the fish are prepared for consumption. We will present how these factors influence risk and, if not incorporated into an assessment, may lead to fish consumption advisories that are not protective to Indigenous communities. We will also demonstrate the importance of characterizing exposure from the consumption of gathered aquatic plants and how these characterizations can impact risk and help inform appropriate cleanup decisions.

**1.06.T-04 A Pathway Towards Equitable and Sustainable Access to Freshwater Resources In Developing Countries**

***Beatrice Opeolu***[1]*, Omoniyi Kolawole Pereao*[1] *and Idris Ayinde*[2]*, (1) Cape Peninsula University of Technology,*

*South Africa, (2) Federal University of Agriculture Abeokuta, Nigeria*
Freshwater resources are needed for domestic, municipal, industrial, and recreational purposes. Global water scarcity is no longer a myth and it is undeniably evident across continents. The incessant droughts in the southern hemisphere especially Africa is also prevalent in the North. Climate change continues to drive freshwater scarcity due to rising temperatures and extreme weather events. Land use practices for economic and social well-being of increasing world population will continue to exert pressure on water resources. Agriculture, manufacturing, mining activities among others do not only use copious amounts of water, they also contribute to the degradation of freshwater ecosystems. These dynamics results in unavailability of water for potable and other uses. A few individuals need large amounts of water to support their lifestyles- gardens, swimming pools, ponds, etc. On the other hand, millions do not have access to the minimum required for food and sanitation due to availability and affordability issues. Water inequality in terms of access and quality is therefore a big issue especially in developing countries. Appropriate water pricing for different uses is therefore critical for equitable distribution to all. Water prices can theoretically be used for several different water management purposes, including generating revenue, scarcity early warning and re-allocation of existing water rights. These in turn, may lead to formulation of policy outcomes, sometimes implying trade-offs between policy objectives. A conceptual framework that will involve all key stakeholders at ecosystem level up to points of water use will be presented. The proposed structure will enhance efforts aimed at water availability and environmental social justice for all in a sustainable way.

**1.06.T-05 Community-based Wetland Health Monitoring Pilot Program using Indigenous Knowledge and Western Science-based Indicators**

*Adi Adiele[1] and **Taylor Lowe**[2], (1) Fort McKay Métis, Canada, (2) Associated Environmental Consultants Inc., Canada*
Fort McKay Métis Nation established a Community-based Wetland Monitoring Program that uses Indigenous knowledge and western science to monitor indicators for wetland health; to develop a program that answers key questions from the community and generates information that can better inform land use planning and decision making; to build community capacity for data collection, reporting and evaluation; create meaningful employment opportunities for community members that includes spending time on the land, to better understand environmental trends in wetland health and ultimately to improve understanding and conditions in key wetlands of concern; and creating opportunities for youth and elders/knowledge to work together to support knowledge transfer and cultural sustainability. Monitoring questions evaluated if wetlands are drying out, if there are fewer healthy wetlands that can be used for traditional harvesting, if it is getting harder to predict how to travel through wetlands, if water is being polluted by nearby industrial sites, and if changes in wetland health are affecting how Fort McKay Metis people use wetlands and how this affects our people and culture. In 2019 FMMN secured funding to establish a 3-year pilot community-based wetland monitoring program. Information was collected for a number of environmental and socio-cultural indicators. The monitoring program was successful in building community capacity for data collection, reporting and evaluation. The active and meaningful participation of community members is a key characteristic of the monitoring program. The Fort McKay Métis have identified community-based monitoring and research as a new way to not only collect scientific data, but also a way to maintain connections to the land, strengthen Métis culture and values such as self-reliance, self-determination, cooperation, faith, happiness, understanding nature, and respect. The Pilot Community-Based Wetland Monitoring Program has created opportunities for youth and elders/land users to work together to support knowledge transfer and cultural sustainability.

**1.07 Nanoparticle Biological Interactions and Their Responses**

**1.07.T-01 Nanoscale sulfur uniquely suppresses fungal disease and increases biomass and yield of crop plants**

***Jason C. White**[1], Yi Wang[1], Wade Elmer[1], Om Parkash[2] and Xing Baoshan[3], (1) The Connecticut Agricultural*

*Experiment Station, (3) University of Massachusetts, Amherst, (4) University of Massachusetts*
Nanoscale sulfur has attracted increased interest as a multi-functional sustainable agricultural amendment to manage plant pathogens, improve crop productivity, enhance nutrition, and prevent heavy metal accumulation. Here, pristine (nS) and surface coated (cS) sulfur nanoparticles were foliar applied or added to soil that was planted with tomato and infested with Fusarium under greenhouse and field conditions. Bulk sulfur (bS), ionic sulfate (iS), and healthy controls treated with the same sulfur containing compounds were included to enable mechanistic understanding. The measured endpoints included time dependent agronomic and photosynthetic parameters, disease severity, yield, and a range of mechanistic biochemical and molecular endpoints, including the expression of 13 genes related to two S bioassimilation pathways and pathogenesis-response, and tissue-specific metabolomic profiles. The impact of treatment on the rhizosphere bacterial microbiome was also evaluated. Disease reduced biomass by up to 87%, but amendment with nS and cS significantly reduced disease progress by 54-56% compared to the infested controls. Increased S accumulation was evident in plant roots and leaves, independent of S type. Molecular analysis revealed particle size and coating specific impacts on the plants. For nS and cS, two-photon microscopy and time dependent gene expression data revealed a nanoscale specific elemental S bioassimilation pathway within the plant tissues. These findings correlated well with a detailed metabolomic profiling of plant tissues at 4, 8, and 16 d which exhibited increased disease resistance and plant immunity related metabolites with nanoscale treatment. The data also demonstrate a time-sensitive physiological window whereby nanoscale stimulation of plant immunity will be effective. An analysis of the rhizosphere soil bacterial community revealed minimal impacts from S treatment. A parallel field study showed that nanoscale sulfur increased the early yield per plant by 54% under diseased conditions, demonstrating that a $33 investment per acre for a form of a required nutrient (cS) led to an increase of $12,200 in economic return per acre in the complete absence of conventional pesticides. These findings provide significant mechanistic insight into novel nanomaterial-based suppression of plant disease, and significantly advances efforts develop sustainable of nano-enabled agricultural strategies for crop protection.

**1.07.T-02 Tuning the Properties of Polymer Nanocarriers for Controlling Foliar Uptake, Translocation, and Biostimulation of Crop Plants**

***Gregory Lowry**, Yilin Zhang and Robert D. Tilton, Carnegie Mellon University*
Extreme climate events are driving the need to boost crop production and resilience. Polymer nanocarriers can delivery climate stress relief agents or nutrients, but these approaches will require efficient translocation of materials from the point of application to selected locations in plants such as roots, stems, leaves, or fruits. However, there are many biological barriers limiting targeted delivery after foliar spray; cuticle, epidermis, cell walls, cell membrane(s). Our work is determining how the properties of polymer nanocarriers can be engineered to efficiently cross barriers to deliver agrochemicals and plasmid DNA, and is developing environmentally responsive carriers that enable stimuli-triggered release of encapsulated agents. Star and bottle-brush polymers are taken up by tomato plants after foliar application with near 100% efficiency. Confocal microscopy, synchrotron X-ray spectroscopy, and ICP-MS are used in combination to quantify translocation and to identify precise locations of the particles in the plants to understand which barriers have been crossed and which are not. A set of thermal- and ROS-responsive polymer carriers can release an encapsulated agent *in vivo* in response to a specific stressor (heat) or through reaction with ROS produced by the plants under stress. Design rules for translocation of nanoparticle carriers in plants are beginning to emerge based on these studies. These rules can inform the design of other nanoparticle-based delivery approaches, and the engineered responsiveness may be leveraged to deliver cargo only after the particles have arrived at the desired location.

**1.07.T-03 Assessing Nano-Induced Sub-Cellular Perturbations of Lithiated Cobalt Oxide Nanomaterials to the Green Algae Raphidocelis subcapitata**

***Eric Ostovich**[1], Austin Henke[2], Curtis Green[2], Robert Hamers[2] and Rebecca Klaper[1], (1) University of Wisconsin, Milwaukee, (2) University of Wisconsin, Madison*
With growing numbers of engineered nanomaterials found in commerce and in the environment, it may not be

feasible to study them all using conventional toxicological methods. And with a lack or toxicity data, there's a need for more nontargeted, high-throughput profiling assays that can characterize biological activity, potency thresholds, and mechanisms of action. In recent years, morphological profiling has shown its ability to provide rich sources of data for interrogating such biochemical perturbations as the morphology of a cell is extremely sensitive and strongly influenced by factors such as metabolism, genetic state, and environmental cues. Additionally, it's been shown that specific biochemical perturbations deliver equally as specific morphological profiles, and therefore any subset of morphological features that deviate from that of healthy cells can serve as a fingerprint to characterize biological activity.

In this experiment, the impacts on algal cell morphology was examined when exposed to the mixed metal oxide nanomaterial, $LiCoO_2$ (LCO). LCO is largely used as a cathode material in lithium-ion batteries and found in many consumer electronics and high-end vehicles. Since LCO has annual productions at high quantities of environmental significance while also lacking infrastructure for recycling and disposal, there is a high potential for environmental release of this material. This is concerning as LCO contains high valence metals with high reactivity and known inherent toxicity. Thus, it will be important to understand how LCO interacts with environmentally relevant organisms that partake in ecosystem dynamics and maintain ecosystem health and sustainability, like green algae.

Cell Painting, a high-throughput morphological profiling assay, was used to visualize different organelles and high-content imaging to obtain a large number of morphological features at the resolution of a single-cell. Different sub-cellular structures of *R. subcapitata*, including chloroplasts, neutral lipid droplets, and nuclei, were analyzed in response to LCO exposure. Statistical differences in each structure were observed between treated vs. non-treated samples, as well as in the overall morphological profile, thus exemplifying the ability of Cell Painting to characterize bioactivities in response to nanomaterials.

**1.07.T-04 Assessment of Multiple Stressors: Combined Effects From Exposure to Zinc-Oxide Nanoparticles and the Pathogen Klebsiella Pneumoniae in Caenorhabditis Elegans**

***Jarad Cochran**, Jason Unrine and Olga V. Tsyusko, University of Kentucky*

In most nanotoxicity assessments, model organisms are exposed to a single stressor, but in nature, organisms are affected by multiple sources of stress, including infections, which might exacerbate or mitigate negative effects of NP exposure. To expand our understanding of the environmental consequences of released NP, this project examined the combined effects of ZnONP on *Caenorhabditis elegans* infected with a common pathogen, *Klebsiella pneumoniae*. Zinc sulfate (Zn ions) and ZnONP exposure decreased reproduction in nematodes, with the $EC_{30}$ of Zn ions and ZnONP being 7.5 ug/mL and 6.5 ug/mL, respectively. Additionally, exposure to *K. pneumoniae* significantly decreased reproduction compared to nematodes fed on non-pathogenic bacteria. To assess the combined stress of ZnONP and *K. pneumoniae*, *C. elegans* were exposed to $EC_{30}$ concentrations of Zn ions or ZnONP along with the pathogen for 8 hours. Unexpectedly, combined exposure to Zn treatment and *K. pneumoniae* resulted in reproduction that was not significantly different from controls. Further experiments aimed to determine mechanisms of the antagonistic effects. Mitigation of the pathogen effects by ionic or particulate forms of Zn is partially explained by the estimates of the colony forming units (CFU) of *K. pneumoniae* within treated nematodes. The nematodes exposed to Zn ions and ZnONP had significantly lower CFU compared to nematodes only exposed to *K. pneumoniae*. Further, our preliminary scanning electron microscopy (SEM) data suggest that zinc adversely affects pathogen morphology in the Zn ion treatment, and this could reduce its pathogenicity. Pathogen biofilm formation was assessed qualitatively and quantitively. Assessments showed that *K. pneumoniae* produces biofilm, even in the presence of Zn (ions or ZnONP); however, Zn exposure significantly lowered biofilm production. Our dissolution and filtration experiments showed that amelioration of Zn toxicity in *C. elegans* by *K. pneumonia*e was not due to dissolved Zn being bound to ligands (>3 kDa) originating from the microbial biomass. Taken together, our results suggest Zn (ions or ZnONP) exposure decreases *K. pneumoniae* ability to form biofilms in host intestines, yet mechanisms of reduction of host Zn toxicity by pathogen exposure remain unknown. Future transcriptomic analyses of these treatments are planned to determine which molecular pathways are involved in the observed antagonistic effects.

**1.07.T-05 Discussion: Nanoparticle Biological Interactions and Their Responses**

***Olga V. Tsyusko***[1]*, Susana Loureiro*[2] *and Elijah Petersen*[3]*, (1) University of Kentucky, (2) University of Aveiro, Portugal, (3) National Institute of Standards and Technology (NIST)*

**1.07.T-06 Toxicokinetics and Bioaccumulation of Silver Sulfide Nanoparticles in Benthic Invertebrates in an Indoor Stream Mesocosm**

***Patricia Silva***[1]*, Ana Rita R. Silva*[1]*, Nathaniel Clark*[2]*, Joanne Vassallo*[2]*, Marta Baccaro*[3]*, Neja Medvešček*[4]*, Magdalena Grgić*[5]*, Abel Ferreira*[1]*, Martí Busquets-Fité*[6]*, Kerstin Jurkschat*[7]*, Anastasios Papadiamantis*[8]*, Victor Puntes*[9]*, Iseult Lynch*[10]*, Claus Svendsen*[11]*, Nico van den Brink*[3]*, Richard Handy*[2]*, Cornelis A.M. van Gestel*[12] *and Susana Loureiro*[1]*, (1)University of Aveiro, Portugal, (2)University of Plymouth, United Kingdom, (3) Wageningen University & Research, Netherlands, (4) University of Ljubljana, Slovenia, (5) Ruđer Bošković Institute, Croatia, (6) Applied Nanoparticles SL, Barcelona, Spain, (7) Oxford University, United Kingdom, (8) NovaMechanics Ltd, Cyprus, (9) Institut Català de Nanociència i Nanotecnologia , Spain, (10) University of Birmingham, United Kingdom, (11) UK Centre for Ecology & Hydrology (UKCEH), United Kingdom, (12) Vrije Universiteit Amsterdam, Netherlands*

Mesocosm studies enable establishment of more realistic scenarios of organism exposure to nanoparticles (NPs), improving evaluation of NPs bioavailability, bioaccumulation and their interactions with biological receptors. Here, a mesocosm test was conducted to assess the toxicokinetics and bioaccumulation of silver sulfide ($Ag_2S$) NPs in a simulated stream environment. The main objectives were to: 1) determine the toxicokinetics of $Ag_2S$ NPs (as model environmentally aged Ag nanoform) and silver nitrate ($AgNO_3$; metal salt control) in the freshwater benthic invertebrates *Girardia tigrina* (planarian), *Physa acuta* (snail) and *Chironomus riparius* (midge larvae), 2) evaluate the potential for Ag bioaccumulation and biomagnification, 3) determine if single-species tests can predict bioaccumulation in the mesocosm test. The study was performed in an indoor modular system with 36 artificial streams, each filled with sediment and artificial pond water. The following species were placed in each unit: *G. tigrina*, *P. acuta*, *C. riparius*, *Lumbriculus variegatus* (oligochaete worm), *Daphnia magna* (water flea) and *Oncorhynchus mykiss* (rainbow trout). Water was spiked daily at a nominal concentration of 10 µg Ag/L. Biota, water and sediment were sampled destructively at days 2, 7 and 14 (last day) and toxicokinetics were determined using one-compartment models. The main findings include the demonstration of the uptake and bioaccumulation of $Ag_2S$ NPs by the three species, suggesting bioavailability of this environmentally relevant Ag nanoform in benthic environments. Dissolved Ag appeared to be more bioavailable for uptake, with higher uptakes and body Ag concentrations determined when exposed to $AgNO_3$. The contribution of different routes of uptake was investigated, and water was the most important Ag exposure medium. No biomagnification of Ag from $Ag_2S$ NP exposure was observed in the food chain snail to planarian, but is likely to occur in the $AgNO_3$ treatment. When comparing mesocosm and single-species test results, the latter generally seemed not able to reliably predict Ag bioaccumulation in the more complex mesocosms. This work allowed the determination of Ag toxicokinetics and bioaccumulation in complex scenarios, including simultaneous exposure routes and species interactions (*e.g.,* predation) that cannot be observed in single-species tests. This study provides ecologically relevant data for use in predictive models to improve risk assessment and regulation of NPs.

**1.07.T-07 An Environmental Hazard Assessment of Nanoscale Exfoliated Graphene and Graphene Oxide: Impacts of Particle Preparation**

***Hans G. Brown***[1]*, Bryan J. Harper*[2]*, Stacey L. Harper*[2]*, Jun Jiao*[3] *and Kaleb Hood*[3]*, (1) University of Kentucky, (2) Oregon State University, (4) Portland State University*

Exfoliated graphene (EG) and graphene oxide (GO) nanoparticles are currently being investigated for a number

of medical and manufacturing purposes. GO has potential for use as a thin-film coating, but requires hazardous reducing agents during preparation. EG may present a safer alternative. In this study, *Daphnia magna*, a common organism used for assessing ecotoxicity in freshwater environments were selected as the model organism. The *D. magna* were exposed to a range of concentrations of EG prepared in n-methyl-2-pyrrolidone (NMP, 0-5 mg/L), EG prepared in 1:1 NMP and ethanol (0-20 mg/L), EG prepared in ethanol (0-24 mg/L), EG prepared in DI-water (0-75 mg/L), GO prepared in ethanol (0-25 mg/L), or GO prepared in DI-water (0-150 mg/L). Mortality and immobility assessments were made at 24 and 48-hrs. The highest levels of mortality were observed in the EG exposures where NMP was used for particle synthesis. The data suggests that ethanol may be a safer preparatory solvent than NMP. Dialysis of prepared particles resulted in a removal of all acute toxicity across both materials and preparatory solvents. With the increased interest in the production of these engineered nanomaterials (ENM), it is essential that an environmental hazard assessment is performed so that new technologies can be designed safer for humans and the environment.

**1.07.T-08 Establishing FAIR use of historical ecotoxicological data for nanomaterial governance**

***Andrew Barrick*** *and Amelie Chatel, Universite Catholique de L'Ouest, France*

Development of novel manufactured nanomaterials is outpacing research capabilities to define environmental risk. Implementing predictive modelling using physicochemical properties is one of the proposed methods to streamline risk assessments for manufactured nanomaterials. The efficacy of this approach hinges on access to robust historical data, which is currently lacking for most ecotoxicological platforms. Centralization of data following FAIR principles can facilitate environmental risk assessment. The aim of NanoInformaTIX is to leverage existing data for mesocosms and use parametric modelling to establish a framework for predicting adverse outcomes. Data from several European research projects were compiled to investigate whether existing data can characterize how physicochemical properties influence bioaccumulative and ecotoxicological potential of manufactured nanomaterials. Results demonstrated an existing need for standardization of reporting for ecotoxicological data. This presentation highlights the integration of FAIR data practices that can be used to augment predictive modelling in ecotoxicology. This presentation highlights current capabilities of nanomaterial ecotoxicity to address 21$^{st}$ century issues and proposes suggestions for FAIRification of ecotoxicological data. This presentation also provides perspectives on database management that maximizes scientific investment for contaminants of emerging concern.

**1.07.P Nanoparticle Biological Interactions and Their Responses**

**1.07.P-We001 Toxic Effects of Binary Mixtures of Metal Oxide Nanoparticles on the Bioluminescence Activity of Pm-lux Recombinant Strain**

***In Chul Kong*** *and Sohyeon Lee, Yeungnam University, Korea, Republic of (South)*

Comparison of the effects of binary mixtures of metal oxide nanoparticles (NPs) on bioluminescence activity was evaluated using $P_m$*-lux* recombinant strain of *Pseudomonas putida mt-2*. Antioxidant activity was measured based on the DPPH (diphenyl picryl hydrazyl) radical scavenging and catalase activity. Different sensitivities and responses were observed according to the type and mixture of NPs. ZnO NP caused the greatest inhibition among the tested NPs. The observed effects of binary combinations of binary NP mixtures were slightly lower than expected, indicating additive mode of action in the mixtures. Results indicated that the DPPH radical scavenging and catalase activity were correlated with the effects of binary NP mixtures on the bioluminescence activity of recombinant strain. More detailed in-depth systematic approaches is needed to evaluate the NPs effect mechanisms involved in bioluminescence metabolic processes, and consideration of chemical mixtures rather than single compounds when evaluating the effects of metal oxide NPs.

**1.07.P-We002 Nanoparticle Surface Chemistry and Morphology Influence Mesophyll Protoplast Uptake and Fate**

***Benjamin Therrien****, Yilin Zhang, Gregory Lowry and Hagai Kohay, Carnegie Mellon University*

Up to 50% of fertilizer and more than 95% of pesticides are wasted because they do not reach their target, resulting in agrochemical runoff and significant environmental deterioration and ecological harm. Food demand is also expected to increase by 50% by 2060. Using nanotechnology to develop more efficient forms of agrochemical delivery is a promising approach to alleviating the environmental damage caused by agricultural runoff and supporting increased food production demand. However, improving nanocarrier design and targeting the carriers to specific location in the plant are key to ensuring its efficacy. Previous studies revealed the roles of nanoparticle (NP) size and charge magnitude in NP uptake and fate in mesophyll protoplasts. This study builds on prior work by examining how NP surface chemistry (Layered double hydroxide NPs with $NH_2$, histidine, PEG, or OH surface groups) and morphology (star polymer sphere NPs, short and long polymer bottlebrush) influence NP uptake and fate in mesophyll protoplasts. Leaf mesophyll cells are extracted from *Arabidopsis thaliana* (Col-0) and exposed to NPs with controlled coatings and morphologies. After NP incubation, protoplasts and free NPs in suspension are separated and the final NP association with protoplasts is evaluated using inductively coupled plasma mass spectrometry. Protoplasts are also imaged using confocal microscopy and synchrotron-based x-ray fluorescent mapping to further examine NP fate and organelle association. Initial results reveal that a smaller NP (5 nm star polymer) does not associate with protoplasts differently than a larger NP of similar charge, material, and morphology (35 nm star polymer), suggesting that NP material and morphology also influence protoplast uptake. We hypothesize that surface chemistries that mimic biomolecules or that disrupt the cell membrane will improve uptake into protoplast. Moreover, we hypothesize that the smallest size dimension will control uptake. Other characteristics in long or rod-shaped NPs, like tensile strength, may also be important. This system serves as a powerful tool for testing NP fate in-vitro and predicting in-vivo fate after foliar application. Advancing our understanding of how NPs interact with the cell membrane enhances our ability to design nanocarriers for agrochemicals and optimize compound delivery.

**1.07.P-We003 Short-Term Exposure to FC60, ZnO and TiO2 Nanoparticles Affects Antioxidant Responses in the Clam Ruditapes philippinarum**

***Davide Asnicar*[1,2]*, Ilaria Marisa[1], Valerio Matozzo[1] and Maria Gabriella Marin[1], (1) University of Padova, Italy, (2) Huntsman Marine Science Center, Canada*

Due to their unique physical properties, nanoparticles have increasingly been produced in recent decades for many industrial and biomedical applications. These products can enter the marine coastal environment through effluents, riverine systems, industrial and urban run-off, representing emerging stressors for organisms. In this work, clams of the species *Ruditapes philippinarum* were exposed for 7 days to three single nanoparticles (fullerene C60, zinc oxide and titanium dioxide) at two environmentally relevant concentrations (1 and 10 μg/L).

At the end of the exposure, a battery of oxidative stress-related biomarkers was measured in gills and digestive gland. The activity of SOD, CAT and GST enzymes was evaluated and the oxidative stress damage in lipids (LPO-TBARS) and proteins (PCC) was quantified.

All biomarkers investigated were altered by the NP exposure. Enzyme activity alterations were detected in both tissues. In particular, CAT activity was the most affected by the exposure to all three NPs. Clams exposed to fullerene C60 showed increased protein damages in the gills, while titanium dioxide caused a significant increase of lipids oxidative degradation in the digestive gland. Zinc oxide did not cause oxidative damages. Limited damage to macromolecules suggested adequate antioxidant defence to protect the animal, at the NPs' concentrations tested at least. Longer-term exposures and exposure to the NPs mixture will help identify whether clams can cope with NPs or will suffer further damages.

Results confirmed the toxicity of these emerging environmental pollutants and the possible risk they pose to marine bivalves, even at the low concentrations tested.

**1.07.P-We004 Effect of anionic nanoclays (LDHs) to Daphnia magna: harmonizing methods to derive reproducible results**

*Fábio Campos, Patricia Silva, Roberto Martins and* ***Susana Loureiro****, University of Aveiro, Portugal*

The expansion of the nanotechnology market may lead to the release of nanomaterials (NMs) into the environment. Consequently, there is increasing need to evaluate their potential risk to the environment and to humans. However, the wide variety and distinct physico-chemical properties of NMs makes their environmental risk assessment very challenging. The standard guidelines currently available do not account for all the nano-specificities, leading to insecurities and uncertainties regarding NM's hazard. Zn-Al layered-double hydroxides (LDHs) are anionic nanoclays with 20 to 40 nm in height, standing as an innovative NM-based solution for antifouling prevention and/or eutrophication mitigation. Such NM properties lead to several challenges when developing strategies to assess their hazard. Considering this, the present study aimed to i) assess the influence of two different OECD-recommended exposure methodologies (serial dilutions of the stock dispersion vs. direct addition of NM powder to each concentration) in the ecotoxicological profile of Zn-Al LDH (bulk powder) to the freshwater species Daphnia magna; ii) use the preferred methodology to evaluate the toxicity of different Zn-Al LDH grain size powders (bulk, <25, 25-63, 63-125, 125-250 and >250 µm) to D. magna. In the direct addition of NM powder methodology, Zn-Al LDH was weighted individually for all target concentrations. For the serial dilution methodology, a stock dispersion was made and used as the highest tested concentration, with the remaining concentrations being achieved by serial dilution. The direct addition of NM to each concentration was deemed as the preferred exposure methodology as the results revealed less variability. For the second aim, the ecotoxicological tests involving the different Zn-Al LDH grain size powders were performed using this methodology. The results showed that different grain sizes caused different toxic effects in D. magna. Some Zn-Al LDH grain size powders are less toxic comparatively to the bulk Zn-Al LDH, posing as an eco-friendlier solution for several applications. Moreover, our findings support Zn-Al LDHs as a less toxic option comparatively to another Zn nanoforms (e.g. ZnO). To conclude, this study provides important data to help harmonizing methodologies for standardized protocols for testing NMs hazard. In the future, interlaboratorial testing must be performed to develop standardized guidelines for ecotoxicological testing with Zn-Al LDHs.

## 1.08 Novel Methods and Approaches for Assessing Effluents and Ambient Water Toxicity

**1.08.T-01 Knowledge Sharing to Improve Toxicity Testing Efficiency and Outcomes: SETAC's Aquatic Toxicity Testing Interest Group and Culture Troubleshooting Subgroup Activities**

*Marcus Bowersox[1],* ***Camille Flinders****[2], James M. Lazorchak[3], Natalie Love[4], Teresa J Norberg-King, David J. Soucek[5] and Trudy Watson-Leung[6], (1) Tetra Tech, Inc., (2) NCASI, Inc., (3) U.S. Environmental Protection Agency, (4) GEI Consultants, Inc., (5) U.S. Geological Survey, (6) Bugsrock Environmental Consulting, Canada*

Toxicity testing has been used globally to evaluate the effects of specific compounds, effluents, and ambient waters on aquatic biota. Standard toxicity testing methods have been developed, which are used for regulatory purposes, monitoring, and Toxicity Reduction Evaluations/Toxicity Identification Evaluations (TRE/TIEs). SETAC's Aquatic Toxicity Testing Interest Group (ATTIG) was initiated to advance the science of aquatic toxicity testing methods, promote improvements and consistency in current testing and culturing protocols, and advance emerging *in vivo* and *in vitro* methods. Consistent and robust toxicity test outcomes depend on test organism culturing practices and adherence to method guidelines. Because educational and training opportunities related to toxicity testing are limited, the ATTIG initiated a Culture Troubleshooting subgroup with the primary objectives of providing a trusted, open, and reliable community for aquatic toxicity professionals and researchers, and a resource repository of aquatic toxicity organism culturing and testing information. Specific focus topics for the working group include method improvements and best practices, performance and sensitivity of test organisms, culture troubleshooting and solutions, method development, test checks, and data and reference toxicant control charting. These collaborative efforts are expected to increase the collective ability to focus on environmental protection by maximizing testing efficiency through consistent culturing conditions across laboratories and providing a platform to address issues pertaining to culturing test organisms used in standardized tests. This presentation will provide an overview of ATTIG activities related to culture troubleshooting, culture challenges associated with non-standard organisms and new method development, and data analyses and sharing. Additionally, a summary of best practices for culture and test performance monitoring parameters, and variability in toxicity estimates associated with reference toxicants will be presented.

**1.08.T-02 Potential risks of pharmaceuticals and personal care products in an urban tributary of the Potomac River**

***Scott Glaberman**, Cheyenne Hawkins and Greg Foster, George Mason University*

Pharmaceuticals and personal care products (PPCPs) are a large group of compounds with diverse chemical structures and modes of action. There is a relative lack of toxicity information of PPCPs compared to highly regulated compounds such as pesticides or legacy chemicals like PCBs. Recently, effects-based methods (EBM) have emerged as one tool to rapidly forecast the potential impacts of emergent micropollutants such as PPCPs. Here we apply an EBM approach to PPCPs in a tributary of the Potomac River in densely populated Northern Virginia within the greater Washington, D.C. Metropolitan Area. Specifically, we pair monitoring data of surface water and sediment from 48 PPCPs from Hunting Creek and the Potomac River near Alexandria, Virginia to high throughput bioassay data in order to forecast risk and specific biological effects. We sampled upstream and downstream of a local water treatment plant (WTP) and near combined sewer overflows (CSOs) and in the main stem of the Potomac River. For surface water, there were 6 chemicals in which the cumulative risk across all sampling sites was greater than 1: ranitidine, tadalafil, carbamazepine, triamterene, caffeine, and DEET. The vast majority of risk derives from the WTP or CSOs. Interestingly, at nearly all sites, carbamazepine, triamterene, caffeine, and DEET show up at some level and contribute to some degree of detectable risk, whereas ranitidine and tadalafil appear to be specifically associated with the sites directly downstream of the WTP. Ranitidine in particular exhibits concentrations near the WTP that are >150x the toxicity value – this is primarily for toxicity effects related to cytotoxicity. Another chemical of potential concern in surface water is carbamazepine. This chemical is the most likely to exhibit endocrine disruption as it is known to be an agonist of the androgen receptor which binds hormones such as testosterone. The risk values for carbamazepine are high (exposure-activity ratios (EARs) >30), and the potential androgenic effects on organisms should be looked at, including sex morphology and reproduction. For sediment, the risk picture looked very different and rather minimal based on available data. Whereas the cumulative EARs across chemicals was nearly 400 for surface water, which is extremely high, EAR values for sediment are less than 1.

**1.08.T-03 Mechanism-based Biomonitoring with Enhanced Throughput for Examination of Human Impacted Surface, Ground and Drinking Water.**

***Jack Vanden Heuvel**[1,2], Megan Granda[1] and Bruce Sherf[2], (1) The Pennsylvania State University, (2) INDIGO Biosciences, Inc.*

Human-impacted surface, ground and drinking water can contain a complex mixture of micropollutants, such as pharmaceuticals, pesticides, and industrial compounds. In vitro bioassays based on various cellular response pathways have been applied to detect and quantify the presence of micropollutants in water samples. These bioassays allow for sensitive, rapid and inexpensive detection of bioactivity while considering complex mixtures of compounds and potential toxicity. However, the current approach where single response pathways are examined sequentially limits its usefulness since it requires dozens of different cell lines and reporter systems and often does not cover a wide range of activities. In these studies, we developed a work-flow to maximize the number of receptors and pathways examined with enhanced throughput and toxicologic information. First, the water sample extract is examined in mixtures of optimized reporter assays that are grouped based on biological niche including: Xenobiotic and Bile Acid Metabolism (XM Panel; CAR2, CAR3, PXR, FXR, VDR, AhR), Lipid and Energy Metabolism (LEM Panel; THRB, PPARA, PPARB, RXRA, PPARG, LXRA); Reproductive and Developmental Effects (EDC Panel; AR, ERA, ERB, PGR, GR, THRA);

Central Nervous System and Basal Metabolism (BM Panel; LXRB, RXRB, RXRG, MR, RARG) and Toxicology and Inflammatory Pathways (TOX Panel; NFkB, NRF2, AP1, p53). Extracts that exhibit positive activity in one of these five panels (representing 28 pathways), can be examined in a dose-response to determine Bioanalytical Equivalent Concentrations (BEQs) for that pathway. In addition, each receptor in the impacted panel can be explored individually to determine its contribution to overall activity. Several environmental and wastewater samples and known contaminants of concern (CECs) have been examined in this manner

**1.08.T-04 Systematic evaluation of factors affecting the characterization of wastewater effluents using gene expression**

***Adam Biales***[1]*, David Bencic*[1]*, Mary Jean See*[1]*, Susan T. Glassmeyer*[1]*, Dana Kolpin*[2]*, Marc Mills*[1]*, Edward Furlong*[2]*, Robert Flick*[1]*, Weichun Huang*[1] *and Tom T. Purucker*[1]*, (1) U.S. Environmental Protection Agency, (2)U.S. Geological Survey*

Wastewater treatment effluent (WWTE) has been identified as a major source of diverse chemical contaminants with the potential to negatively affect the function of receiving ecosystems. Though the toxicity of effluent is dependent on the individual components of the mixture, it is difficult to predict toxicity due to incomplete characterization of the total chemical composition, the influence of the non-chemical background, and the potential for chemical components to interact and alter their respective bioactivity. For these reasons, there has been an increasing focus on the development of effect-based measures that can account for these complicating factors to characterize the total bioactivity of the effluent mixture. Several research efforts have employed gene expression-based approaches with the aim of characterizing effluent. Generally, these efforts identify genes that are differentially expressed relative to either an upstream reference site or laboratory exposed organisms and bioactivity is interpreted through mapping of these genes to annotated biological pathways. However, rarely do gene expression results corroborate the results of paired analytical chemical analysis. There are likely a myriad of reasons for this, but it is noted that there have been few, if any, systematic evaluations of the factors that can affect nature of gene expression in field applications. The current work aimed to address this knowledge gap by systematically evaluating the stability of gene expression to characterize the bioactivity of a wastewater treatment effluent over repeated sampling events taken twice per day (morning and evening) over two days at the same locations (upstream and in the effluent), using different control conditions (laboratory water vs. upstream reference), and across different sampling strategies (organisms deployed in the sampling locations concurrent with grab sampling vs laboratory organisms exposed to grab samples). To the best of our knowledge, this will be the most comprehensive evaluation of gene expression in field applications to date and has the potential to help guide future efforts to reduce background noise and to maximize the interpretability of gene expression-based measures.

**1.08.T-05 Biological Activity in U.S. Food Processing Plant Effluent**

***Elizabeth Medlock Kakaley***[1]*, Nicola Evans*[1]*, Brett R. Blackwell*[1]*, Rachel Hofer*[1]*, Laura Hubbard*[2]*, Paul M. Bradley*[2]*, Dana Kolpin*[2] *and Dan Villenueve*[1]*, (1) U.S. Environmental Protection Agency, (2) U.S. Geological Survey*

Commercial food, beverage and feedstock processing facilities produce wastewater with complex mixtures and are a probable source of bioactive contaminants, but current monitoring of these wastewaters is primarily focused on basic constituents including pH, dissolved oxygen, and suspended solids. We measured organic compound (Phase I:580, Phase II:700) concentrations and biological activity in a two-phase study of wastewater effluent from 23 food processing facilities accross the United States. Samples were assessed for estrogen, androgen, glucocorticoid, and peroxisome proliferator-activated (PPAR; α and γ) receptor activity and for activation of 24 additional nuclear receptors (Attagene Trans-FACTORIAL) and 52 transcription factor signatures (Cis-FACTORIAL). Effluent samples from each site (23 of 23) contained estrogenic activity (0.05 – 1.62; median: 0.26ng E2Eq/L) and 10 of 23 contained estrogenic activity levels that may cause adverse effects in aquatic species after chronic exposure. Androgenic activity was detected in 9 of 23 effluent samples (0.19 – 8.41; median: 0.32ng DHTEq/L) and no glucocorticoid or PPAR α/γ activity was detected in any sample above method detection limits (MDL). Attagene analysis corroborated single endpoint bioassay results indicating estrogenic activity in a variety of effluent types. To further characterize environmental exposures in Phase II, effluent, along with stream water up and downstream from outfall, bed sediment, and aquatic organisms were collected from a subset of Phase I sites (7 of 23). Samples up and/or downstream from an ethyl alcohol manufacturing and soybean/oilseed processing facility produced estrogenic activity comparable to effluent concentrations. Samples downstream from a non-poultry meat processing and soybean/oilseed processing facility produced androgenic activity comparable to effluent concentrations. No biological activity was detected above MDL in sediment samples. Known estrogen and androgen chemical concentrations quantified using targeted analytical methods (HPLC/MS-MS) did not completely explain measured biological activity, confirming the added value of bioassays in water quality screening applications. Future aquatic-species tissue analysis will provide insight on potential exposure effects to detected mixtures of biologically active contaminants. Abstract does not necessarily reflect US Environmental Protection Agency views or policy but does represent the views of the US Geological Survey

**1.08.T-06 Florida Apple Snail (Pomacea paludosa)-an ecologically relevant and sensitive species for aquatic toxicity testing**

***Tham C. Hoang***[1]*, Gary M. Rand*[2]*, Paul Hitch*[3] *and Nichole Taggart*[3]*, (1) Auburn University, (2) Florida International University, (3) Loyola University Chicago*

The Florida apple snail (*Pomacea paludosa*) is a freshwater gastropod mollusk native in Florida, which inhabits a variety of freshwater habitats in Alabama, Georgia, Louisiana, and South Carolina. In Florida, especially in the Everglades ecosystem, *P. paludosa* is an important species and serves as the main food resource of higher trophic species, including the snail kite. We have conducted toxicity studies with *P. paludosa* for over 15 years and found *P. paludosa* to be a valuable test species to support the results of other standard species in acute and chronic aquatic toxicity studies. This species can also be used to determine uptake and bioconcentration of pollutants from water, sediment, and food. *P. paludosa* is as sensitive to pollutants as other standard species that have been used in toxicity testing, such as fathead minnow.  Under similar water quality conditions, copper (Cu) and zinc (Zn) LC50s for *P. paludosa* and fathead minnow were similar. Among freshwater snail species that have been used for research, *P. paludosa* has been reported to be more sensitive to metals (i.e., Zn) than 80% of freshwater snails. In addition, *P. paludosa* has been found to accumulate high metal concentrations, such as up to 2,800 mg/kg Cu. This is the highest Cu accumulation in living organisms that can be found in the literature. The latter result suggests *P. paludosa* to be a suitable bioindicator species for monitoring metal pollution. *P. paludosa* has also been reported to ingest microplastics at concentrations up to more than 1,000 microplastics/snail. This is the highest microplastic accumulation in aquatic organisms reported in the literature. The lung feature of *P. paludosa* allows them to survive out of water for a short period of time. This feature can support growth studies, where snails can be collected for growth measurements over time and returned to experimental chambers after measurement without harmful affect to them. *P. paludosa* can also be cultured in the laboratory with less maintenance than other standard species for aquatic toxicology testing. Our research to determine the optimal water quality conditions for *P. paludosa* under water flow-through and static-renewal systems found similar growth and reproduction rates. Our research findings support using *P. paludosa* for aquatic toxicology research, especially for the U.S. southern region, when effluents, metals, pesticides, and microplastics are under consideration to determine their potential toxicity.

**1.08.T-07 Strategies for Toxicity Identification Evaluations when Standard Tests and Species do not Resolve the Toxicity**

***Konrad Kulacki****, William L. Goodfellow and Alexandra Steele, Exponent*

Effluent discharges from municipal and industrial wastewater treatment systems have been traditionally monitored for chemical constituents. However, many effluents contain countless chemicals in the mixture. A substantial limitation to using only chemical specific monitoring is that one needs to know or suspect the chemicals that may be in the effluent to be evaluated, or a standard list of chemicals is necessary to ensure the regulatory compliance net is sufficiently broad. To overcome this limitation, the uses of whole effluent toxicity testing (WET) or whole effluent assessment (WEA) can determine whether an effluent as a whole is toxic to aquatic organisms. Traditional methods use standard test species such as fish, invertebrates, and plants (e.g., algae) to assess whether effluents are unacceptably toxic. The use of the biological organism as the analytical detector makes sure that toxicants do not pass into receiving waters, even when the chemical is not immediately known. In many instances, even if investigations characterize the effluent as toxic, it is difficult to identify or remove the toxicant. Should effluent samples be either acutely or chronically toxic, an additional strategy termed toxicity identification evaluation (TIEs) is often utilized. TIEs use physical and chemical fractionation procedures to further characterize toxicity, isolating and identifying the specific toxicant(s) in the effluent. When standard test strategies do not adequately resolve the toxicant in the effluent, non-standard species or novel tests can be used in the assessment of effluents. This presentation will explore strategies to further evaluate effluents when standard species or test strategies do not adequately characterize or identify the toxicant.

**1.08.P Novel Methods and Approaches for Assessing Effluents and Ambient Water Toxicity**

**1.08.P-Th028 Comparison of Zebrafish Toxicity Between Different Developmental Windows of Exposure to Three Environmentally Relevant PFAS Compounds**

***Prarthana Shankar***[1]*, Jenna E. Cavallin*[1]*, Michael Edward Ellman*[2]*, Brett R. Blackwell*[1]*, Sara M. Vliet*[1]*, Carlie LaLone*[1]*, Daniel L. Villeneuve*[1] *and Steven Lasee*[1]*, (1) U.S. Environmental Protection Agency, (2) Oak Ridge Institute for Science & Education at US EPA*

The per- and polyfluoroalkyl substances (PFAS) are of significant global concern due to their highly ubiquitous and persistent nature, bioaccumulation in organisms, and potential toxicity. While studies have identified harmful effects of PFAS, little is known about how varying exposure length impacts chemical hazard. Zebrafish have emerged as a sensitive and high-throughput model for measuring toxicity associated with chemicals, including PFAS, in a relatively short span of time. The current study's objective is to leverage zebrafish to explore effects of exposure to PFOS, PFOA, and PFHxS for 14 exposure lengths between 6 and 144 hours post fertilization (hpf). Zebrafish were exposed to eight concentrations of each PFAS chemical (0-100 uM), chlorpyrifos (positive control for behavior), and 0.33% DMSO (vehicle control) for varying lengths of time starting at 6, 24, 48, or 96 hpf, and ending at 24, 48, 96, 120, or 144 hpf. Morphology was assessed daily, zebrafish neurobehavioral responses to alternating dark and light cycles were measured at 120 hpf for windows ending at 120 or 144 hpf, and RNA was collected at the end of each window to investigate whole-animal transcriptomics. Daily 50% chemical renewal was conducted, and media was analyzed by liquid chromatography-mass spectrometry at each window's beginning and end to validate exposure concentrations. Exposures ending at 24, 48 or 96 hpf did not lead to overt toxicity for any of the PFAS tested. When zebrafish were exposed to 100 uM PFOS, except for 96-120 hpf, significant mortality was observed at all exposure windows ending at 120 hpf. For windows 6-144 and 24-144 hpf, significant mortality at 144 hpf was identified at the two highest PFOS concentrations (100 and 35.16 uM). No significant mortality was observed following exposure to PFOA, PFHxS, or any other PFOS concentration. Currently, we are analyzing zebrafish morphology (hatching, growth, and swim bladder inflation) and behavior responses, and evaluating transcriptomic points of departure (tPOD) from whole-animal RNA. Results of this study will provide information on 1) the hazard to zebrafish associated with varying exposure lengths to select PFAS chemicals, and 2) the temporal variability associated with tPODs estimated under various exposure scenarios, specifically different exposure durations and at different zebrafish developmental stages. *Contents of this abstract neither constitute, nor necessarily reflect, official USEPA policy.*

**1.08.P-Th029 Spatial and Temporal Distribution of Glucocorticoid and Estrogen Receptor-Mediated Bioactivities in Relation to a Wastewater Input on the South Platte River (Denver, CO, USA)**

***Jenna E. Cavallin***[1]*, Catherine A. Adams*[2]*, Larry Barber*[3]*, Brett R. Blackwell*[1]*, Paul M. Bradley*[3]*, Alexander R.*

*Cole[4], Drew R. Ekman[1], Steffanie Keefe[3], Kristen Keteles[1], Julie Kinsey[1], Kristen M. Romanok[3], Kelly L. Smalling[3], Emma Stacy[1], Dana Winkelman[2] and Daniel L. Villeneuve[1], (1) U.S. Environmental Protection Agency, (2) Colorado State University, (3) U.S. Geological Survey, (4) Baylor University*
Previous studies on the South Platte River near Denver, Colorado, USA have detected in vitro bioactivity, including estrogen receptor (ER) and glucocorticoid receptor (GR) activity, at some of the highest levels in surface waters of the U.S. Building upon previous research, the present case study demonstrates how new approach methodologies (e.g., in vitro bioassays) can be employed in biological effects-based evaluations of environmental field samples. Surface water samples were collected for two studies including temporal and spatial evaluations. For the temporal evaluation, total ER and GR agonist bioactivities were determined over the course of winter and spring 2022, the time period with the lowest river flow and corresponding greatest percent effluent contribution to the South Platte River system. Surface water samples were collected monthly at three South Platte River sites located around a major wastewater treatment plant (WWTP) to capture temporal (monthly) variations in the bioactivities: upstream, proximal to the discharge, and 1.5 km downstream from the discharge. A more spatially intensive study was conducted during November 2021 in which water samples were collected at 12 sites over an 80 km reach of the South Platte River to determine how far downstream of the WWTP the biological activities could be detected. GR-mediated bioactivity remained around 40 ng dexamethasone equivalents/L as far as ~30 km downstream, before dropping about 60% over 8 km and diminishing thereafter. The bioeffects-based data, along with targeted analytical chemistry data, will be compared with hydrological/wastewater modeling aimed at predicting the concentrations of chemicals in wastewater impacted stream systems. These data will be used to help define the temporal and spatial scope of potential adverse effects associated with ER- and GR-mediated bioactivities in the hydrologically complex South Platte River system. *The contents of this presentation neither constitute, nor necessarily reflect, US EPA policy.*

**1.08.P-Th030 Ecotoxicological Evaluation of Water and Sediment of the Doce River and Marine Areas Impacted by the Fundão Tailings Dam Failure, Brazil**

***Camila Martins***[1]*, Taciana Miranda[1], Cassia da Silveira[1], Regina Rola[1], Josiane da Silva[2] and Thays Guedes[1], (1) Federal University of Rio Grande, Brazil, (2) Fundação Espírito-santense de Tecnologia, Brazil*
The collapse of the Fundão Dam (Minas Gerais, Brazil), occurred in November 2015, was the largest environmental disaster in the world mining industry. This episode, released approximately 55 million cubic meters of waste containing heavy metals and other stressors related to mine tailings, deeply impacted the Doce River basin and the Atlantic Ocean adjacent to the Doce River mouth. As part of a monitoring program established to determine the impacts of the dam failure on aquatic biodiversity, the present study investigated the ecotoxicological effects of the mine tailings spill, through laboratory toxicity tests (acute and chronic) using organisms of different trophic levels as bioindicators. A set of tests carried out with each sample was used to classify the environments according to the degree of toxicity presented, ranging from non-toxic to highly toxic. Water and sediment conditions were evaluated over 3 years (2018 to 2021), during dry and rainy seasons. Samples of water collected in Doce River were considered as non-toxic or slightly toxic, while the sediment varied between non-toxic and moderately toxic. A temporal analysis shows a reduction in toxicity at the mouth of the Doce River over the monitoring. However, studies performed in the marine area, show that both water and sediment are highly impacted in the regions near to the mouth of the Doce River, including an environmental protection area. Regardless the site and season of sampling, toxicity of sediment was greater than water, possibly due to the higher content of organic matter, an important metal binder. Highest toxicity values were recorded for the tests performed with organisms belonging to the zooplankton that reinforces their sensitivity and use for environmental diagnosis. In addition, Principal Component Analysis indicated relationships between the metals typical of the tailings and the toxicity of the samples. Therefore, results presented above indicate that even 6 years after the dam rupture, the monitored environments, mainly marine, are still impacted.

**1.08.P-Th032 Behavior of Adults and Juveniles of a Freshwater Shredder Shrimp, Xiphocaris elongata, After Individual Exposure to Insecticides**

***Marla Valeria Santos-Crespo*** *and Omar Pérez-Reyes, University of Puerto Rico*

The function and vitality of Caribbean insular streams are governed by shrimp. Shrimp are essential in organic matter processing, control of primary productivity through consumption, and as food. Their response to important emerging contaminants that reduce water quality, such as pesticides, is yet to be understood. Behavior and toxicity data is mostly available for the model organisms from the temperate region. This project describes the individual exposure of adults and juveniles of the freshwater shredder shrimp *Xiphocaris elongata*, endemic and ubiquitous to the Caribbean, to the neonicotinoid insecticides thiamethoxam and imidacloprid. Shrimp behavior was assessed through video recording after exposure to each insecticide. Adult shrimp showed lethargy in their response to physical cues, while juvenile shrimp showed hyperactivity. Such a change in behavior may reduce the shrimp's ability to 1) control their movements during foraging, and 2) evade predators. It is key to continue to describe the repercussions that the persistent use of pesticides can have in tropical ecosystems, giving insight to stakeholders on how they can development pesticide regulation and monitoring.

**1.08.P-Th033 Influence of Behavior Protocol Light Intensities on Determining Chemical Effects**

***Bridgett Hill***[1]*, Deborah Hunter*[2]*, Kimberly Jarema*[2]*, Zachary Rowson*[1]*, Bridget Knapp*[1]*, Jeanene Olin*[2] *and Stephanie Padilla*[2]*, (1) Oak Ridge Institute for Science and Education (ORISE), (2) U.S. Environmental Protection Agency*

The U.S. Environmental Protection Agency is evaluating new approach methods to screen and prioritize chemicals for developmental neurotoxicity. Analysis of larval zebrafish behavior is recognized as a higher throughput testing strategy to identify developmentally neurotoxic chemicals. Interpretation and comparison of behavioral data is challenging, and some of the complexity results from inconsistent approaches (e.g. chorion status, age at time of behavior testing, and presence/absence of chemical) in the light/dark transition test between laboratories. Here we focus on one variable, the light phase intensity of the behavior protocol, and its influence on the ability to detect chemically induced behavioral effects. Six days post fertilization, normally developed zebrafish larvae were acutely exposed to a chemical previously shown to induce hypoactivity and then their behavior was assessed in a light/dark transition assay. Testing consisted of two different phases: forty minutes of a light phase at either 335 or 3,500 lux light intensity, followed by forty minutes of a dark phase (12 lux light intensity). The control (*i.e*., untreated) larvae generally had higher activity under the higher light phase intensity (3,500 lux), particularly in the light phase. While there was an overall significant difference in the activity patterns between the two light phase intensities, the chemical effects were comparable: all concentrations were different from the control independent of the light phase intensity used for the light/dark transition test. Despite testing with two markedly different light phase intensities, the same conclusion can be drawn. Here we sought to answer the question of whether a lower light phase intensity impedes the ability to detect chemically induced behavioral effects. We found there to be a baseline control difference using the two light phase intensities selected, but with the one chemical that was tested, we were still able to detect a difference in behavioral toxicity in both scenarios. Regardless of the results with this specific test chemical, it's important to measure and report the light intensity used in order to develop more globally harmonized protocols. *This abstract does not reflect US EPA policy.*

**1.08.P-Th034 Effects of Exposure to Binary Mixtures of Thiamethoxam and Imidacloprid on the Neurobehavior and Development of Fathead Minnow Larvae**

***Anya Johanna Jeninga***[1]*, Sara Duffy*[1]*, Elisabeth Harrahy*[2] *and Tisha King-Heiden*[1]*, (1) University of Wisconsin, La Crosse, (2) University of Wisconsin, Whitewater*

Neonicotinoid insecticides have emerged as contaminants of concern due to their frequent application and persistence within the environment. While designed to be selective for insect nicotinic acetylcholine receptors (nAChRs), recent studies indicate they cause subtle neurobehavioral toxicity in fish. Thiamethoxam (THM) and imidacloprid (IMD) are commonly found together in surface waters in the Central Sands region of Wisconsin at levels exceeding ecological thresholds. The aim of this study was to evaluate the effects of exposure to mixtures of THM and IMD on development and behavior of fathead minnow (*Pimephales promelas*). Fish were exposed to a 1:1 mixture of THM and IMD (0, 0.02, 0.2, 2, 20, 200 µg/L) shortly after fertilization via waterborne exposure with 100% daily renewal for 8 days. Effects on survival, hatching, growth, embryonic motor activity, and predator escape response will be compared to other experiments where fathead minnows were exposed to THM or IMD alone. Preliminary findings suggest that co-exposure to both THM and IMD do not lead to additive toxicity. On-going analyses regarding the impacts of exposure to mixtures of neonicotinoids in fish may be useful for setting water quality guidelines in Wisconsin.

**1.08.V Novel Methods and Approaches for Assessing Effluents and Ambient Water Toxicity**

**1.08.V-01 Using Imidacloprid to Evaluate a Novel Reproductive Toxicity Test Method for Hyalella azteca**
***Hufsa Khan***[1]*, Ryan S. Prosser*[1] *and Adrienne Bartlett*[2]*, (1) University of Guelph, Canada, (3) Environment and Climate Change Canada*
*Hyalella azteca* is a freshwater benthic crustacean used in ecotoxicology because it is ubiquitous in North American freshwater systems and is sensitive to changes in water quality. The standard reproduction toxicity test method for this species outlined by Environment and Climate Change Canada (ECCC) is a 6-week sediment test that begins with juveniles. Reproductive endpoints typically occur in weeks 4-6 of the standard test. It is difficult to achieve robust data for reproduction in *H. azteca* because there is naturally a high biological variability associated with reproductive yield and because effects on reproduction often co-occur with effects on growth.

The purpose of this study was to create a novel reproduction toxicity test method for *H. azteca* to improve the quality and quantity of reproductive data generated. Four-week, water-only, static-renewal reproduction tests were initiated using sexually mature (6-7-week-old) organisms in a 3 male : 7 female sex ratio, based on the results of a previous study that determined the optimal sex ratio of *H. azte*ca to reduce variability in brood size. Standard 6-week reproduction toxicity tests using juvenile amphipods were adapted to water-only, static-renewal conditions, and were completed so the reproduction data could be compared between test methods. For both methods, amphipods were exposed to sub-lethal concentrations of Imidacloprid (0.33, 1, 3, 9 ug/L, determined from previous studies), and exposure solutions were renewed weekly, at which point survival and reproduction were assessed. Growth was measured at the end of the tests. Comparisons between methods will be focussed on reproduction, but will also include assessments of survival, growth, and amount of resources invested. Results will reveal if the novel reproduction test method will improve toxicological assessments using *H. azteca*.

**1.08.V-05 Applicability of passive sampling and bioassay measurements in assessing performance of water treatment technologies: A case study of the South African landscape**
*Ratanang P.V. Mlaba, Thabo T.I. Nkambule,* ***Hlengilizwe Nyoni*** *and Tshepo J Malefetse, University of South Africa*
Use of chemical analysis alone is not sufficient to protect water systems from chemical pollution. A more holistic approach that accounts for the effect of chemical mixture is necessary to provide a more realistic base for water quality assessment, monitoring and management. Recently, increased attention has been put on the employment of in vitro or in vivo toxicity tests together with chemical analysis methods. Thus, in this work we report efforts made in the application of a different battery of bioassays targeting different toxicological pathways, in evaluate the toxicological and ecotoxicological risks of treated wastewater effluents, surface waters and reclaimed waters for direct potable reuse based on effect-based methods and passive sampling technologies. Prospects of combining toxicity profiling with integrative passive sampling techniques, such as (1) Silicon rubber (SR); (2) Membrane Assisted Passive Sampler (MAPS), (3) Chemcatcher and (4) Polar Organic Chemical Integrative Sampler (POCIS) and its applicability for water quality assessment was explored.

The extracts from passive sampling devices were used for extensive chemical analyses and battery of in vitro and in vivo bioassays covering a range of toxicological endpoints. The decision on the use of SR; MAPS and POCIS devices was to cover the entire range of organic contaminants than can be obtained with a single sampler. Detection and quantitation of sequestered contaminants was accomplished using LC-MS and GC-MS. Cytotoxicity of water extracts and fractions was successfully determined using V-1 and MDA-kb2 cells plated in 16 well in Dulbecco's Modified Eagle Medium (DMEM) modified with 10% dextran-coated charcoal (DCC) serum at a concentration of 1 x 104 cells per 100 ul. Cell growth was monitored using the xCELLigence real-time cell analyzer (RTCA).

## 1.09 Scientific Advances in PAH Research Enabled by Superfund Research Centers

### 1.09.T-01 Source Apportionment Of Polycyclic Aromatic Hydrocarbons In The Sediment Of The Newtown Creek Superfund Site

***Mohson Al Hello**[1], Mahdi Chitsaz[1], David R. Burris[2] and Lisa A. Rodenburg[1], (1) Rutgers University (2) Crooked Creek Environmental*

Newtown Creek is a Superfund Site located in New York City that is impacted by PAHs due to oil spills, industry, and urban runoff. Fifty Polycyclic Aromatic Hydrocarbons (PAHs) were measured in over 1,000 samples of surficial sediment and sediment cores from this site as part of the Superfund process. This data set was examined using Positive Matrix Factorization to identify PAH sources. The PMF analysis isolated six factors (NC1 through NC6) that represented different types of sources. NC1 and NC5 were similar to coal-related sources, with NC1 representing the low molecular weight (MW) fraction and NC5 representing the high MW fraction. This splitting of the coal signal probably reflects the greater tendency of the low MW fraction to dissolve, volatilize, and biodegrade. These processes have altered the coal signal so extensively that PMF recognizes the low MW fraction as its own factor. NC2, NC3, and NC4 represented petrogenic sources. NC2 was most similar to the PAH profile of crude oil, while NC3 was most similar to diesel and NC4 was most similar to fuel oil. NC6 was a high MW pyrogenic profile that probably represents urban background and PAHs from urban runoff. The spatial and temporal variations in the abundance of these six factors were examined to identify potential sources.

### 1.09.T-02 Firefighter Dermal Exposure Assessment with Silicone Samplers

***Emily Bonner**[1], Gavin Horn[2], Denise Smith[3], Steven Kerber[2], Kenneth Fent[4], Lane Tidwell[1], Ricky Scott[1], Kaley Adams[1] and Kim Anderson[1], (1) Oregon State University, (2) Underwriters Laboratories, (3) Skidmore College, (4) NIOSH*

Epidemiology studies have demonstrated elevated cancer rates for structural firefighters and a high percentage of cardiovascular events during or following firefighting activity. Polycyclic aromatic hydrocarbons (PAHs) are generated during combustion and are recognized as carcinogens and are implicated in cardiovascular disease progression and events. In this intervention study, commercially available firefighter personal protective equipment (PPE) typical of the modern US fire service was compared to an intervention configuration of PPE with a one-piece liner to eliminate interfaces at the waist and neck. Mannequins (n=16) dressed in the PPE ensembles were placed in a Fireground Exposure Simulator for 12 min with a couch as a fuel to mimic chemical exposures during a residential fire. Silicone samplers were placed outside of the PPE in the chamber to measure air concentrations. Silicone samplers were also worn by mannequins under the PPE at the neck, chest, and wrist to passively sample organic chemicals that broke through the PPE during the burn scenarios. Mannequins wearing the two PPE configurations were paired by co-location in the chamber; four total burns were conducted, with two mannequin pairs in each. All silicone samplers were analyzed with gas chromatography, tandem mass spectrometry for 63 different parent and alkylated PAHs. The list of PAH analytes includes and exceeds the EPA's current list of 16 priority PAHs, which is the current standard for evaluating firefighter exposures. 51 of these analytes were detected in at least one sample in the study, 9 of which have not been previously reported in fireground exposure studies. Paired t-tests with a Benjamini-Hochberg correction were used to compare co-located mannequin samples at the neck, chest, and wrist, for sum concentrations of low and high molecular weight PAHs (2-3 or 4-7 rings respectively). There is moderate statistical evidence that low molecular weight sum concentrations at the neck (p=0.001) and chest (p=0.015) and high molecular weight sum concentrations at the chest (p=0.020) are higher under the standard PPE than the intervention PPE after the simulated burns. Furthermore, exposures at the neck were generally greatest, and exposures at the wrist were the lowest for both types of PPE. Based on this study, firefighter dermal protection could be improved with the implementation of a physical barrier at the interfaces of the PPE, such as the one-piece liner tested

**1.09.T-03 Detection of PAH compounds in DWH crude oil and their effects on Caenorhabditis elegans germ cell apoptosis, associated with CYP450s upregulation**

***Xiaoping Pan*** *and Joseph Ryan Polli, East Carolina University*

The Deepwater Horizon (DWH) oil spill marked the largest environmental oil spill in human history, where it was estimated a large amount of the polycyclic aromatic hydrocarbons (PAHs) were released with crude oil into the environment. In this study, common PAH compounds were quantitatively determined in crude oil from the DWH spill by gas chromatography-mass spectroscopy (GC-MS). Twelve PAH compounds were identified and quantified from a 100× dilution of DWH crude oil: naphthalene (7800 ng/mL), acenaphthylene (590 ng/mL), acenaphtehen (540 ng/mL), fluorene (2550 ng/mL), phenanthrene (2910 ng/mL), anthracene (840 ng/mL), fluoranthene (490 ng/mL), pyrene (290 ng/mL), benzo(k)fluoranthene (1050 ng/mL), benzo(b)fluoranthene (1360 ng/mL), dibenz(a,h)anthracene (2560 ng/mL), and benzo(g, h, i) perylene (630 ng/mL). Toxicity assays using the nematode, *Caenorhabditis elegans* (*C. elegans*), indicated a single PAH compound from oil, naphthalene, increased germ cell apoptosis; thereby, may adversely affect progeny reproduction. The number of apoptotic germ cells significantly increased from 1.4 to 2.5 when worms were treated with 10 μg/mL of naphthalene and from 1.3 to 2.5 and 3.5 cells in presence of 1 μg/mL and 5 μg/mL of benzo(a)pyrene, respectively. Five CYP450 genes (*CYP14A3, CYP35A1, CYP35A2, CYP35A5,* and *CYP35C1*) were significantly upregulated following 500× dilution of dispersed crude oil exposure ($p < 0.05$). These results suggest that CYP450s may play a role in bioactivation of PAHs in crude oil, resulting in DNA damage-associated germ cell apoptosis.

**1.09.T-04 Role of Alkylated Polycyclic Aromatic Hydrocarbons in Mixture Toxicity from a Legacy Creosote Site**

***Ian Moran***[1]*, Robyn Leigh Tanguay*[1]*, Katrina M Waters*[1,2] *and Kim Anderson*[1]*, (1) Oregon State University, (2) Pacific Northwest National Laboratory*

Creosote is a pesticide used to preserve wood products. Typically derived from distillation of coal tar, creosote is a complex mixture containing mostly polycyclic aromatic hydrocarbons (PAHs) and their derivatives. While creosote is a common contaminant, the toxic effects of weathered creosote are poorly understood. In particular, alkylated PAHS are abundant constituents of creosote and many petroleum products and are known to become enriched relative to their respective parents through weathering. Less is known about the toxicity of alkylated PAHs than their parent compounds. Despite this, alkylated PAHs have been shown to contribute substantially to the toxicity of PAH mixtures in the environment. The goal of this study is to understand the contribution of alkylated PAHs to the toxicity of a complex, weathered mixture from a legacy creosote site. This study utilizes low density polyethylene passive samplers deployed at a former wood treatment facility to accumulate freely dissolved organics in the surface water. Passive samplers are extracted and analyzed by gas chromatography - tandem mass spectrometry for unsubstituted and alkylated PAHs. To assess toxicity, embryonic zebrafish are exposed to passive sampler extracts in 96-well plates and are observed at 24 and 120 hours post fertilization for a suite of behavioral and morphological endpoints. A twelve-month sampling campaign at the site has demonstrated substantial temporal variability in chemical abundance and toxicity with sum PAH concentrations in two adjacent months varying by greater than a factor of two and LC50 values varying by a similar magnitude. Alkylated PAHs constituted the majority of measured PAHs in all samples (83-89%). Representative mixtures containing the most abundant unsubstituted PAHs failed to replicate the toxicity of whole passive sampler extracts. This suggests that compounds other than routinely monitored unsubstituted PAHs are driving toxicity at this site. Additionally, mixture interactions appear to mediate the observed toxicity in zebrafish. Ongoing work seeks to determine the drivers of toxicity and the role of alkylated PAHs by fractionation of field collected mixtures in an effects-directed analysis framework. Understanding the role of alkylated PAHs and their interactions with other mixture constituents can inform remediation efforts and improve our ability to protect human health and water quality.

**1.09.T-05 Time-related Alteration of Aqueous-Phase Polycyclic Aromatic Hydrocarbon (PAH) Photoproducts in the Presence of TiO2 Nanoparticles**

***Lindsey St. Mary***[1,2]*, Lisandra Trine*[2]*, Courtney Roper*[3]*, Staci Simonich*[2]*, Martin McCoustra*[1]*, Theodore Henry*[1] *and Jackson Wiley*[2]*, (1)Heriot-Watt University, United Kingdom, (2)Oregon State University, (3) University of Mississippi*

Polycyclic aromatic hydrocarbons (PAHs) and titanium dioxide ($TiO_2$) nanoparticles (NPs) are photoactive environmental pollutants that contaminate aquatic environments. Aqueous-phase interactions between PAHs and $TiO_2$-NPs are of interest due to their emerging environmental relevance, particularly with the deliberate application of $TiO_2$-NPs to remediate pollution events (i.e., oil spills). Investigations into distinct PAH photoproduct formation and PAH/$TiO_2$-NP photocatalytic interactions can provide important new information on the environmental and health impacts resulting from these complex photochemical processes. Our objective was to investigate anthracene (ANT), benzo[a]pyrene (BaP), fluoranthene (FLT), phenanthrene (PHE), and pyrene (PYR) photoproduct formation following UVA (320-400nm) irradiation in the presence and absence of $TiO_2$-NPs. Distinct PAH solutions were prepared alone or in combination with $TiO_2$-NPs in OECD medium, UVA irradiated, and either exposed to larval zebrafish or collected for chemical analyses of hydroxylate PAHs (OHPAHs) and oxygenated PAHs (OPAHs). The expression profiles of genes encoding for enzymes involved in PAH biodegradation showed PAH-specific and time-dependent inductions that demonstrated changes in PAH and photoproduct bioavailability in the presence of $TiO_2$-NPs. Chemical analyses of PAH/NP solutions in the absence of larval zebrafish identified and quantified diverse OHPAH and OPAH photoproducts of differing size and ring arrangements, which suggested photodissociation, recombination, and ring re-arrangements of PAHs occurring either during or following UVA irradiation. All five PAHs investigated showed heightened oxidative potential following irradiation, but $TiO_2$-NP and time-related increases were PAH-specific. The exploitation of biological responses and analytical chemistry provided novel insights into distinct PAH photochemistry, $TiO_2$-NP influence on photoproduct formation and bioavailability, and the significant role time plays in PAH-related photochemical processes within the aquatic environment.

**1.09.T-06 Exposure to Benzo[a]pyrene (BaP) Induces Gut microbiota Shifts and Developmental Toxicity in Zebrafish (Danio rerio)**

***Prabha Ranasinghe***[1]*, Nishad Jayasundara*[1]*, Tara Catron*[2]*, Akila Harishchandra*[3] *and Richard Di Giulio*[1]*, (1) Duke University, (2) BASF Corporation, (3) University of Maine*

Chemical exposures can disturb the gut bacterial community structure that may modify toxicity outcomes in an organism. Chemical-induced perturbations alter the bacterial diversity, metabolic pathways, and organismal physiology, but shifts in microbial diversity that links to these changes remain critical knowledge gaps. To address this, we investigated the consequences of early life alteration of gut commensal bacteria on energy metabolism, neurodevelopment, and behavior following exposure to BaP. Zebrafish embryos at 1 day post fertilization (dpf) were reared conventionally (CR), microbe free axenic (AX), or axenic colonized on day 1 with adult zebrafish gut homogenate (AXC). They were then exposed to 0, 5 and 10 µM of BaP (at 1dpf) and reared until 6 dpf. Our results demonstrated differential toxicity trends for all the analyzed endpoints in microbe free axenic cohort (AX) compared to the two colonized cohorts (CR and AXC). Metabolic rate was increased with the BaP exposure in the AX cohort, whereas decrease in metabolic rate was observed in colonized cohorts. AX larvae demonstrated hypoactivity with BaP exposure whereas colonized larvae demonstrated the hyperactivity with the BaP exposure. Similarly, Dose dependent decrease in Gamma-aminobutyric acid

(GABA) levels were observed in AX cohort, but opposite trend was observed for the CR and AXC cohorts. 16S rRNA amplicon sequencing analysis indicated impacts of BaP on gut community structure with potential functionality changes that may explain the observed phenotypic variations in BaP treated groups based on the colonization status. Further, histological observations indicated possible inflammation in the BaP exposed groups in AX larvae compared to CR and AXC larvae. Collectively, these data suggest that shifts in gut microbiota is linked to prominent differences in gut epithelial inflammation and may modify the chemical toxicity and the organismal physiology.

**1.09.T-07 Benzo[a]pyrene Exposure Induces Persistent Transgenerational Mitochondrial Dysfunction in Zebrafish**

***Lindsay Jasperse****, Jordan Kozal and Richard Di Giulio, Duke University*

At the Duke University Superfund Research Center (DUSRC), we aim to understand the developmental impacts of environmental toxicants, with an overarching theme of “early life exposure, later life consequences”. We are particularly interested in how evolutionary adaptations to PAHs driven by multigenerational exposures influence ecological risk assessments for Superfund sites. We seek to better understand the fitness costs associated with multigenerational exposures to PAHs, including remediation scenarios, in which offspring are raised in uncontaminated environments. Our work has a particular focus on mitochondrial effects, as mitochondria are known targets of PAHs and mitochondrial function is intricately linked to organismal bioenergetics and behavior. This study builds on previous work by our lab which demonstrated that chronic (21 day) dietary BaP exposure in adult female F0 zebrafish (*Danio rerio*) impaired ATP-linked respiration and mitochondrial coupling efficiency in F1 (maternally exposed) and F2 (germline exposed) embryos and caused hypoactivity in F1 larvae. We extended this study through an F5 generation to determine whether BaP-induced mitochondrial dysfunction during development can persist through generations without germline exposure. To assess mitochondrial function in developing zebrafish, we utilized the powerful technology of the Agilent Seahorse XFe24 Extracellular Flux Analyzer to measure oxygen consumption rates in real time in live 24 hours post-fertilization (hpf) embryos. Mitochondrial respiration was altered in the F3, F4, and F5 generation embryos from BaP-exposed lineages, including decreases in ATP-linked respiration and coupling efficiency. Behavioral toxicity of 6 days post-fertilization (dpf) zebrafish larvae was assessed in collaboration with the DUSRC Neurobehavioral Toxicity Assessment Core, utilizing the DanioVision platform paired with EthoVision tracking software to quantify larval swimming during an alternating light/dark test. Hypoactivity was observed in the F3, F4, and F5 generation larvae from BaP-exposed lineages. This work demonstrates that mitochondrial dysfunction persists across multiple unexposed generations, and likely contributes to the multigenerational toxicity of PAHs. These data can help inform more accurate ecological risk assessments for Superfund sites, highlighting the importance of continuing to assess organism fitness post-remediation.

**1.09.T-08 Transcriptomic and Methylomic Changes Underlying Evolved Polycyclic Aromatic Hydrocarbons Resistance in Teleost Fish**

***Akila Harishchandra**[1], Richard Di Giulio[2] and Nishad Jayasundara[2], (1) University of Maine, (2) Duke University*

Evolved resistance to chemical contaminants is a unique ecological outcome of anthropogenic pollution and remains an important consideration in risk assessment. The physiological mechanisms underlying vertebrate chemical resistance is complex and remain poorly understood. The Atlantic killifish (*Fundulus heteroclitus*) in the Elizabeth River (ER), VA have evolved resistance to extreme levels of persistent organic pollution (Polycyclic Aromatic Hydrocarbons (PAHs)). The Aryl Hydrocarbon Receptor (AHR) has been identified as a key regulatory pathway underlying Atlantic killifish PAH adaptation, and studies show that the evolved resistance is at least partly genetic, though there could be more novel molecular mechanisms that are remained uncovered. Here, we analyzed high throughput hepatic RNA-seq and Reduced Rapid Bisulfite Sequence (RRBS) data of two killifish subpopulations inhabited in Republic (RP-pollution tolerant), and Kings Creek (KC-pollution sensitive) sites to uncover the molecular mechanisms reflected in the transcriptomic level and the corresponding epigenomic signals, underlying PAHs resistance. We found 835 and 308 genes that were differentially expressed (DEGs) and methylated (DMGs) respectively in RP fish compared to KC fish. Except for the AHR2b gene (downregulated in RP fish), none of the AHR pathway genes were differentially expressed indicating that the AHR pathway is repeatedly desensitized in the pollution-tolerant killifish subpopulation. Intronic methylation has been detected in the majority (252) of the DMGs, including intronic hypermethylation in the AHR2b gene. Intron hypermethylation and the downregulation of the AHR2b gene in RP fish could likely be signaling a novel molecular mechanism underlying PAHs tolerance, but further experiments are required to warrant it. The gene ontology enrichment analysis found that the functional genes of metabolic biosynthetic processes were downregulated in RP fish, postulating the likelihood of killifish endocrine system processes disruption when evolving PAHs resistance. However, further experiments are required to confirm whether this disruption was caused by the PAHs or other chemicals. Overall, this study confirms the implication of the AHR pathway and posits some novel mechanisms (e.g., endocrine disruption) underlying Atlantic killifish pollution resistance. Further studies are underway to compare methylomic and transcriptomic data with genomic data from the two populations of these fish.

## 1.09.P Scientific Advances in PAH Research Enabled by Superfund Research Centers

### 1.09.P-Th035 Rapid, Low-Cost Measurement of PAH Contamination in Oysters Using Novel Antibody-Based Biosensor Technology

***Kristen Prossner****, Ellen Harvey, George Vadas, Michael Unger and Hamish Small, Virginia Institute of Marine Science (VIMS) and William & Mary*

Sessile, filter feeding bivalves such as oysters can readily bioaccumulate environmental contaminants like polycyclic aromatic hydrocarbons (PAH) freely dissolved in water. With a limited metabolic capacity, oysters lack an efficient means to excrete PAH. While this characteristic presents an important public health risk via consumption of contaminated seafood, it also makes oysters valuable biomonitoring organisms. Conventional analytical methods for measuring PAH contamination in seafood are time-consuming and expensive, limiting their utility in time sensitive events (i.e. oil spills and floods) or for widespread environmental monitoring. Current screening methods intended to prioritize samples for more extensive analyses are unreliable. A highly sensitive biosensor method has been developed to measure total 3-5 ring PAH concentration in small volume (1-2 mL) aqueous samples in minutes using a monoclonal PAH antibody. Concentrations measured using this method correlate well with results of conventional GC-MS analysis. With these features, the biosensor has shown great promise as a screening tool to measure PAH contamination in oysters and was used to rapidly and inexpensively measure PAH concentration in oysters throughout the Elizabeth River watershed in southeastern Virginia (USA): an urban estuary impacted by legacy creosote contamination and home to Atlantic Wood Superfund site. While both native oyster populations and transplanted aquaculture oysters are used for biomonitoring, PAH concentrations accumulated over time differ between these groups leading to a discrepancy as to which group accurately reflects the level of bioavailable PAH at a contaminated site. To explore why differences may exist, PAH accumulation was measured over time in native, transplanted diploid, and transplanted triploid oysters using the biosensor during a 6-week field experiment. Additionally, via a novel immunohistochemical application, the PAH antibody was used to visualize and localize accumulation of complex environmental PAH mixtures in specific tissues with confocal microscopy. With the ability to measure concentrations in near real-time at a fraction of the cost compared to conventional GC-MS, biosensor technology shows promise as a powerful screening tool to improve human health risk assessments as well as environmental monitoring and remediation efforts at contaminated sites.

### 1.09.P-Th037 Benzo[a]pyrene-Induced Impacts on Paternal Genotype Result in Multigenerational Gene Expression, DNA Methylation, and Behavioral Changes

***Mary Caroline Sturgis***[1]*, Zacharias P. Pandelides*[1]*, Cammi Thornton*[1]*, Neel Aluru*[2] *and Kristine L. Willett*[1]*, (1) University of Mississippi, (2) Woods Hole Oceanographic Institution*

The objectives of this study were to determine the sex-dependent contributions to developmental and behavioral effects of preconceptional exposure to benzo[a]pyrene (BaP). BaP is implicated in numerous adverse outcomes in offspring of exposed parents, but the molecular mechanisms for the developmental and multigenerational effects associated with BaP exposures have yet to be fully elucidated. 5D zebrafish were fed 1% of their bodyweight of 708 ± 24 μg BaP/g food twice per day, resulting in 14 μg BaP/g fish/day for 21 days. Fish were then spawned using a crossover design, resulting in F1 cohorts of control and three crosses from BaP-exposed parents. Behavioral effects were measured at 96 hpf in the F1 fish using the light:dark assay. Significant hyperactivity was observed in both crosses from BaP-exposed male parents. Using an open field test, female F1 adult offspring from the BaP male x BaP female cross were also significantly hyperactive. To assess parental sex-dependent molecular mechanisms that could be contributing to toxicity, gene expression and DNA methylation changes in F0 sperm and eggs and 10 hpf F1 embryos were measured using RNAseq and reduced representation bisulfite sequencing (RRBS), respectively. Embryos resulting from the BaP male and control female cross had the most differentially methylated regions (DMRs) and differentially expressed genes. Some DMRs were associated with genes encoding chromatin modifying enzymes suggesting regulation of chromatin conformation by DNA methylation. Parental dietary BaP exposure caused persistent behavioral changes wherein the male germline contributed most significantly to the multigenerational adverse outcomes. Research supported by NIEHS 1R21ES030154.

### 1.09.P-Th038 Concentration-Response Gene Expression Analysis in Zebrafish Reveals Transcriptional Responses Phenotypically-Anchored to Retene Teratogenicity

***Lindsay B. Wilson**[1], Ryan S. McClure[2], Katrina M. Waters[2], Michael T. Simonich[1] and Robyn Leigh Tanguay[1], (1) Oregon State University, (2) Pacific Northwest National Laboratory*

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants and are associated with human disease. Canonically, many PAHs induce toxicity via activation of the aryl hydrocarbon receptor (AHR) pathway. While the interaction between PAHs and the AHR is well-established, understanding which AHR-regulated transcriptional effects directly result in observable phenotypes and which are adaptive or benign is important to better understand PAH toxicity. Retene is a frequently detected PAH in environmental sampling and has been associated with AHR2-dependent teratogenicity in zebrafish. To interrogate transcriptional changes causally associated with retene teratogenicity, we conducted whole-animal RNA sequencing at 48 hours post-fertilization after exposure to eight retene concentrations. The concentrations were selected to produce effects ranging from no phenotype to mortality and malformations in 100% of animals at 5 days post-fertilization. We identified a concentration-response relationship between retene teratogenicity and differential gene expression in both number of DEGs and magnitude of expression change. Genes differentially expressed at only non-teratogenic concentrations were enriched for transforming growth factor-β (TGF-β) signaling pathway disruption while DEGs identified at only teratogenic concentrations were significantly enriched for response to xenobiotic stimulus and reduction-oxidation reaction activity. DEGs which spanned both non-teratogenic and teratogenic concentrations showed similar disrupted biological processes to those unique to teratogenic concentrations, indicating these processes were disrupted at low exposure concentrations. Gene co-expression network analysis identified several gene modules, including those associated with PAHs and AHR2 activation. One, Module 7, was strongly enriched for AHR2-associated genes and contained the strongest responses to retene. Benchmark concentration (BMC) of Module 7 genes identified a median BMC of 7.5 μM, nearly the highest retene concentration with no associated teratogenicity, supporting the hypothesis that Module 7 genes are largely responsible for retene teratogenicity.

### 1.09.P-Th039 Examining the Effect of Aqueous Benzo(a)pyrene Exposure on Anxiety-like Behavior in the Zebrafish, Danio rerio

***Alicia Dunton**, Gil Martinez Bautista and Warren W. Burggren, University of North Texas*

Benzo(a)pyrene (BaP) is a ubiquitous environmental toxicant found in anthropogenic mixtures such as crude oil, air pollution, vehicle exhaust, and in some natural combustion reactions. Yet, we understand surprisingly little of BaP's acute and chronic impacts on animals. Aquatic animals such as fish may encounter BaP through road runoff and oil spills, but few studies have examined the impact of aqueous exposure on adult fish, and fewer have examined the resulting fitness-relevant behavioral consequences and their long-term persistence. We sought to target this by examining how water exposure to BaP influences anxiety-like behavior in zebrafish. Fish were exposed for 24 hours to 0.5, 5 and 50 uM of BaP in standard aquarium water, and anxiety-like behavior was assessed using a light/dark preference test (LDPT) and a novel tank diving test (NTD). Time spent in dark portions of the LDPT and on the bottom of the tank in the NTD indicated anxiety-like behavior. Initial testing was performed immediately after exposure, and then repeated one week and one month after exposure to determine persistence. Histological analysis was performed on all groups at all time points to determine general neuronal presence and apoptosis. Immunohistochemistry was performed to determine presence of dopaminergic neurons. Based on previous research in rodent models, we hypothesized that BaP would increase anxiety-like behavior in zebrafish immediately following exposure, and that these changes would persist over the one-month testing period. Additionally, we hypothesized that overall neuronal presence and dopaminergic neuronal presence would decrease as a result of BaP exposure. Understanding changes in anxiety-like behavior from BaP exposure may give insight into how populations of fish explore and interact with their environment and with predators, and how these interactions persist even when toxicants are no longer present.

**1.09.P-Th040 Assessing the Acute Toxicity of Photodegraded Naphthalene in a Dermal Model**

***Molly Brzezinski****, Wei Xu and Kayla Simpson, Texas A&M University, Corpus Christi*

Polycyclic Aromatic Hydrocarbons (PAHs) are a diverse group of pyrogenic and petrogenic chemicals that result from wild-fires, automobile exhaust, and oil refining processes. Naphthalene is one of 16 priority PAHs that the US Environmental Protection Agency has identified for their bioaccumulation and carcinogenic effects in occupational settings. Naphthalene has also been shown to readily photodegrade into other potentially harmful intermediate compounds when exposed to sunlight for periods of time. Though there is evidence that sunlight can enhance the toxicity of these chemicals in aquatic organisms, the toxicity of photodegraded naphthalene has not been studied in a human dermal model. Therefore, the objective of the present study was to assess the toxic effects of naphthalene after 2, 4, 16, and 24 hours of photodegradation on cell viability, migration capacity, and epithelial thickness with HaCaT human keratinocytes. Results revealed that 16-hour photodegraded treatments had negative effects on cell viability (84.2% survival) compared to naphthalene alone (100.2%). Cell migration was assessed with a scratch assay and demonstrated that 4-hour and 16-hour photodegradation treatments negatively impacted gap closure, with most severe effects in 16-hour treatments. Three-dimensionally stratified HaCaTs were analyzed for epithelial thickness, and our results show decreased thickness in 16-hour photodegraded treatments (11.2µm) compared to control samples (17.6µm). These results suggest that photodegradation affects the toxicity of naphthalene which will be validated in a murine model.

**1.09.V Scientific Advances in PAH Research Enabled by Superfund Research Centers**

**1.09.V-01 Optimized GC/MS Analysis of PAHs with Hydrogen Carrier Using a Novel EI Source**

*Bruce D. Quimby and* ***Anastasia Alekseyevna Andrianova****, Agilent Technologies, Inc.*

Recent concerns with the price and availability of helium have led laboratories to look for alternative carrier gases for their GC/MS and GC/MS/MS systems. For GC/MS, hydrogen is the best alternative to helium. A novel EI source for GC/MS and GC/MS/MS was developed and optimized for use with hydrogen carrier gas. The novel source reduces undesirable in-source reactions, provides improved spectral fidelity and chromatographic peak shape. This presentation discusses an instrument configuration and operating conditions that provide a robust means of analyzing PAHs in difficult matrices with hydrogen carrier gas.

The method was optimized using the novel EI source, midcolumn backflushing, the appropriate choice of column dimensions, liner, collision cell gas flow, and collision energies. This resulted in very good peak shape and excellent linearity across the calibration range from sub-pg to one ng level. System precision and robustness were demonstrated with replicate injections of an extract from a high organic content soil. Excellent results were obtained with both with Single Quadrupole and Triple Quadrupole GC/MS systems. The added selectivity of MS/MS compared to MS simplified data review.

## 1.10.P The Microbiome: An Emerging Tool for Predictive Ecotoxicology

### 1.10.P-Mo021 Evaluating metagenomic analyses for undercharacterized environments: what's needed to light up the "dark matter"?

***Kelsey Thompson***[1]*, Will Nickols*[1]*, Lauren J McIver*[1]*, Yancong Zhang*[2]*, Eric A. Franzosa*[1] *and Curtis Huttenhower*[1]*, (1) Harvard T.H. Chan School of Public Health, (2) Broad Institute of MIT and Harvard*
Shotgun metagenomics can be applied to deeply characterize microbial communities in a wide variety of environments. However, a gap remains in the interpretation of environmentally-sourced communities, since they regularly contain more, more complex, and less well-characterized organisms than better-studied ecologies such as the human microbiome. This occurs for several reasons, including the density of phylogenetically diverse organisms in these environments; their individual genomic diversity; and the relative lack of characterized reference isolates. We thus assessed the impact these factors have on a variety of typical, state-of-the-art metagenomic analysis methods. Comparing multiple reference- and assembly-based methods resulted in a wide range of sensitivity and specificity for taxonomic detection when applied to real and synthetic environmental communities. While taxonomic profiling methods typically agreed on the most abundant organisms, they disagreed on the identity, number, and abundances of rarer microbes. This was in part due to differences among taxonomic databases, but more often attributable to underlying algorithms. As expected, assembly-based methods performed very poorly on environmental samples with lower sequencing depth, but at higher depths identified community components omitted by all reference-based methods. Assembly-based methods thus dominate recall at higher sequencing depths, but with results that are often difficult to contextualize. Reference-based profilers can achieve high specificity, but at the cost of sensitivity; sensitivity remained highest in sequence classification methods, but these in turn particularly lacked specificity. This highlights opportunities for both data and algorithm development for environmental metagenome interpretation, and the need to more closely integrate host- and non-host-associated microbial community research.

### 1.10.P-Mo023 Multi-omics approach for correlation analysis between pathogenic symptom, pathogen, and metabolome of Neopyropia tenera

***Hyeonjeong Bae*** *and Tae-Yong Jeong, Hankuk University of Foreign Studies, Korea, Republic of (South)*
Laver, also called by nori, zicai, or gim has been one of the major harvestable and edible marine algae in East Asia and the global yield from aquaculture has increased gradually. The mainly used genera of red algae for cultivation are *Neopyropia* and *Neoporphyra.* For the last decades, the outbreaks of related red algal diseases caused by fungi-like oomycetes, bacteria, viruses, or diatoms have been reported continuously, however, red algal diseases are poorly understood. In this study, we analysed the correlation between disease symptom occurrence, microbiome species and abundance, and metabolic regulation of a laver, using thalli of *Neopyropia* sampled from aquaculture in West Sea of South Korea. Microbiome and metabolome of the randomly sampled *Neopyropia* at the designated region during the winter harvesting season were treated for further analyses of MS-based metabolomics and 16S and 18S rRNA gene amplicon sequencing. For searching biomarker candidates along health status of *Neopyropia*, each randomly selected thallus was scored by the size of symptoms (diseased spots and discolored spots) and surface bacteria and metabolome of the same thallus were measured by MiSeq system and LC-QTOF-MS.

We anticipate that associated bacteria and metabolites vary depending on the health status of thallus, and annotated metabolites reveal the impact on metabolic pathway. As a result, amplicon sequencing is expected to confirm that several known pathogens, oomycete *Pythium* species, *Pontisma* (formerly *Olpidiopsis*) species, and several bacteria for red-rot disease, *Olpidiopsis* disease, and ana-aki disease, respectively. Additionally, unexpected genus will be likely revealed that are related to the symptom of the disease.

This study fills the gaps on links between known and unknown pathogens and red algae symptoms. More importantly, this study will be the basis of further study for searching biomarkers indicating marine environmental change including pathogenic conditions to seaweeds.

**1.10.P-Mo024 Effects of Erythromycin on Juvenile Rainbow Trout Microbiome and Fitness**
***Phillip Ankley***[1]*, Jonathan Karl Challis*[1,2]*, Jenna Cantin*[1]*, Katherine Raes*[1]*, Emily Kennedy*[1]*, Yuwei Xie*[1]*, Markus Hecker*[1]*, Markus Brinkmann*[1] *and John Giesy*[2]*, (1)University of Saskatchewan, Canada, (2) Agriculture and Agri-Food Canada (AAFC), Canada*
Antibiotics are continuously discharged via wastewater effluent in urban environments including erythromycin, a commonly used broad-spectrum macrolide antibiotic in human medicine. Discharged antibiotics and their pseudo-persistence could potentially result in negative effects on ecosystem health, including the condition and health of exposed fishes. Gut microbiomes of fish serve central roles in the overall condition of hosts, and gut microbiome dysbiosis could have important implications for health of host fishes. A 7-d dietary exposure of erythromycin followed by a 7-d depuration period was conducted to understand the acute effects of erythromycin on juvenile rainbow trout gut microbiome and host health. Three levels of exposure (0.1, 10, and 1000 µg/g) and four collection time points (4-d, 7-, 11-d, and 14-d) were utilized for the study to investigate responses of juvenile rainbow trout using 16S rDNA amplicon sequencing of gut microbiome and mRNA-seq of host gut tissue. Bile samples were analyzed for changes in chemical concentrations to understand uptake and depuration of the antibiotic, with highest treatment having detectable levels of parent compound with levels dropping quickly at 11-d followed by no detection at 14-d. Minor differences were seen in changes in weight and Fulton's condition factor for juvenile rainbow trout throughout the experiment. Erythromycin was hypothesized to decrease abundances of microbes and shift gut microbiome community composition of exposed fish, while mRNA-seq was anticipated to provide changes in gene expression leading to altered pathways using gene enrichment analyses. Future steps include the measurement of short-chain fatty acids in collected plasma samples using target metabolomics to understand functional changes to the gut microbiome. Results of this study provide insights into potential effects of changes in microbiome communities and impacts on the intestinal tissue due to antibiotic exposure, leading to altered fitness.

**1.10.P-Mo025 Effects of Cotton Farming on Prokaryotic Microbiota in an Abundant Passerine Host**
***Serguei V Drovetski***[1]*, Gary Voelker*[2]*, Kenan O. Matterson*[1] *and Natalie K. Karouna-Renier*[1]*, (1) U.S. Geological Survey, (2), Texas A&M University*
Agrochemical pollution associated with modern crop production poses an imminent threat to biodiversity, surpassing that of global climate change. Grassland birds sharply declined in the USA and Canada since pesticide use became routine in the 1960s. This decline is much steeper than declines of avian abundance observed in other North American biomes. Wildlife mortality events caused by exposure of non-target animals to lethal doses of pesticides are infrequent. In contrast, sublethal exposure to pesticides is ubiquitous and has been linked to chronic fitness consequences and population declines over time. Pesticides deplete vertebrate food resources and adversely affect animal physiology and health. Our recent study on prairie grouse (presented at SETAC 2020) showed that exposure to crop production results in dysbiosis of the cecal microbiota and elevated load and richness of virulence factors and antibiotic resistance genes. Prairie grouse are ground-dwelling primary consumers that spend considerable amounts of time at crop fields, which increases their agrochemical exposure risk. Furthermore, cropland-foraging grouse are deprived of fruit and ground dwelling insects that dominate diets of birds residing in uncultivated grasslands. Grouse ceca function as fermentation chambers and retain digesta for up to 24 hours (~50 bacterial generations) that allows microbiota to adapt to available resources thereby reducing variation in the community structure relative to other parts of the gut, which have much shorter digesta retention time. In this study we focus on ileal microbiota of an abundant,

primarily insectivorous passerine that has mostly arboreal lifestyle and lacks developed ceca – the northern mockingbird (*Mimus polyglottos*). We utilize shotgun metatranscriptomics to test whether we can detect effects of exposure to cotton farming on ileal microbiota of the northern mockingbird similar to those observed in prairie grouse ceca. We sampled mockingbird populations residing in areas with and without cotton production in Texas and compared richness and composition of their prokaryotic microbiota and virulomes. Our data demonstrate that exposure to cotton production appears to be associated with reduced prokaryotic richness, changes in prokaryotic community composition and elevated virulome richness and load. In contrast to prairie grouse ceca, however, these trends had marginal statistical support reflecting much greater variation among individuals.

**1.10.P-Mo026 Zooplankton and Associated Microbiome Response to Simulated Oil Spill and Remediation Efforts**

***Phillip Ankley***[1], *Yuwei Xie*[1], *Lauren Timlick*[2], *Madeline Stanley*[3], *Markus Brinkmann*[1], *Markus Hecker*[1], *John Giesy*[1] *and Vince Palace*[2], *(1) University of Saskatchewan, Canada, (2) IISD Experimental Lakes Area (IISD-ELA), Canada, (3) University of Manitoba, Canada*

Oil spills can disturb aquatic ecosystems with best practices for the restoration of impacted environments continually improving. Nutrient enrichment and floating wetlands can stimulate microbial degradation of petroleum constituents assisting in the recovery of impacted aquatic ecosystems. Zooplankton provide essential functions to aquatic ecosystems and can serve as useful indicators of ecological health while zooplankton-associated microbiome and their responses to oil remediation practices are largely unknown. In this study, we applied DNA and RNA COI and 16s rDNA metabarcoding to profile the zooplankton and associated microbiome response to simulated oil spills and select remediation practices. The objectives of the project were to assess the response of zooplankton and associated microbiome using alpha and beta diversity metrics and compare DNA and RNA metabarcoding to measure change in the respective communities to ecosystem perturbations. Model oil spills of conventional heavy crude were applied to isolated shorelines in a pristine boreal lake, and following primary recovery efforts, two remediation practices were employed which included enhanced monitored natural recovery and engineering floating wetland. Five sampling time points were taken over the summer of 2021, including one pre-exposure sampling time point in June and sample collection every following month. The overall dominant zooplankton genera included *Bosmina*, while the dominant prokaryote family was *Comamonadaceae*. Differences in RNA and DNA alpha and beta diversity profiles existed between zooplankton and associated microbiomes, while RNA and DNA exhibited similar results for alpha diversity response to treatments for prokaryotes, but not for zooplankton. Overall, engineered floating wetlands had the largest negative effect on alpha diversity for the zooplankton-associated microbiome. Future steps include additional analyses using both RNA and DNA community profiles, comparison of zooplankton amplicon sequencing with morphological data, incorporation of environmental chemistry data, and a recovery time point (summer 2022).

**1.10.P-Mo028 Microbial Communities on the Skin and in the Gut of the Gulf Killifish (Fundulus grandis) Exposed to Differing Levels of Polycyclic Aromatic Hydrocarbons**

*Deborah Carr, James Carr, Ezinne Osuji and* ***Alejandra Jacquez****, Texas Tech University*

In April of 2010, the Deepwater Horizon oil rig located off the coast of Louisiana exploded leading to the release of over 130 million gallons of crude oil into the waters of the Gulf of Mexico. Research has shown that a group of chemical compounds present in oil, polycyclic aromatic hydrocarbons (PAHs) can alter biological processes in organisms exposed to the types of pollutants including the microbiome. The gut microbiome is involved in a variety of biological functions which include immune and hormonal signals, regulation of feeding and digestion. Disruptions to this homeostasis can tell us fate or response of organisms exposed to certain pollutants. Among the organisms affected by the oil spill are Gulf Killifish (*Fundulus grandis*), a species that can be found across the Gulf of Mexico and as such a model organism for studies of this nature. Gut and skin samples taken from fish collected in areas affected by oil pollution will be analyzed through 16S rRNA metagenomic sequencing. Fish were collected from Barataria Bay, Louisiana, a location characterized as highly impacted by the DWH spill, and from a reference site in Port Arthur, Texas. I hypothesize that gut microbial assemblages will differ in both richness and evenness between oiled and non-oiled sites for both skin and gut samples in *F. grandis*. There have not been many studies addressing gut microbiomes of Gulf Killifish in association with varying levels of PAH exposure. This study will help advance the knowledge of the long-lasting effects of catastrophic and unprecedented events on marine ecosystem, such as the Deepwater Horizon oil spill.

**1.10.P-Mo030 Florfenicol in salmon diet: microbiome and fish gut changes**

***Giovanna Monticelli***[1]*, David Peggs*[2]*, Carlos Zarza*[2]*, Joseph H. Bisesi Jr.*[3]*, Jason Tyler Magnuson*[1]*, Daniel Schlenk*[4] *and Daniela Maria Pampanin*[1]*, (1 )University of Stavanger, Norway, (2) Skretting Aquaculture Research Centre, Stavanger, Norway, (3) University of Florida, (4) University of California, Riverside*

The use of antibiotics to treat various fish diseases in aquaculture is still necessary, and dosages and treatment lengths greatly vary depending on local regulations. Although concerns about the impacts of antibiotics in aquatic organisms are reported worldwide, the potential adverse effects on fish gut microbial communities and fish health are still not well known. In this study, changes in the fish gut microbiome and the transcriptome due to treatment with florfenicol were evaluated. Adult Atlantic salmon (*Salmo salar*) were treated with commercial feed coated with florfenicol, at different industry-relevant doses: 10 mg/kg (low dose), 20 mg/kg (medium dose) and 30 mg/kg (high dose) for 18 days, followed by a recovery period of 15 days post-treatment. Gut tissues were sampled for mRNA-sequencing analysis to quantify the gene expression in both the host gut as well as the gut microbiome. Metatranscriptomics results showed 3319, 3124, and 3082 unique differentially expressed genes (DEGs) in the low, medium, and high dose treatments, respectively. The overall gene expression was similar in the medium and high dose treatments after 18 days of exposure; however, DEGs differ in the two groups after the recovery period. In the fish transcriptome, approximately 35000 DEGs were identified. *In silico* pathway assessments of the fish transcriptome, using Ingenuity Pathway Analysis (IPA), showed that the florfenicol treatment primarily induced alterations in expression levels of genes associated with DNA replication and apoptosis in all the treatments and that the identified pathways predicted gut inflammation and gastrointestinal disease. In addition, many of the identified pathways (26%) were found exclusively in the low dose treatment. Pathways identified in the high dose predicted activation of xenobiotic metabolism, which persisted in the recovery period. Overall, antibiotic administration caused a dose-dependent change in the fish gut microbiome, preventing a return to the same conditions after the recovery period. The impacts of these changes on fish health require additional study.

**1.10.V The Microbiome: An Emerging Tool for Predictive Ecotoxicology**

**1.10.V-01 Impact of Disinfectants on Pseudomonas aeruginosa and Staphylococcus aureus Dual Species Biofilms**

***Richard Melton*** *and Krassimira Hristova, Marquette University*

Since the start of the COVID-19 pandemic, the world has seen a significant increase in the use of antimicrobials, specifically disinfectants in the built environment. It is essential to understand how disinfectants use will affect the diversity and resistance profiles of indoor microbial communities. The bacteria found in these environments are often in biofilms. Previous work has shown that in the presence of disinfectants, bacteria exhibit increased rates of resistance evolution and many show co-resistance to other disinfectants and antibiotics. However, most of this research has been done with single species or with mixed planktonic cultures. Our objective is to establish a dual species biofilm model to better understand the impact of disinfectants on antimicrobial resistance and virulence. Our data show that bacteria in biofilms are significantly more resistant to disinfectant treatment compared to the same species grown planktonically. We have established a dual species model using continuous flow biofilm bioreactor inoculated with both *Pseudomonas aeruginosa* and *Staphylococcus aureus.* Initial data from the bioreactor shows that we are able to keep both bacteria at a constant relative abundance for several days with a slight dominance of *Pseudomonas aeruginosa*. The addition of the disinfectant Benzalkonium Chloride (BAC) has an impact on the relative abundance of these bacteria within the biofilm. We also observed an increase in the BAC minimum inhibitory concentration (MIC) of these bacteria when grown in dual specie culture compared to the single species biofilm MIC, indicating that growth in polymicrobial environment protects the target organism by several orders of magnitude from the disinfectant. Continued use of this model will allow us to better understand how bacterial species interact under selective pressure and what are the long-term effects on bacterial virulence and on the evolution of antimicrobial resistance. The overall results might have a significant impact on choosing strategies for disinfection in the build environment.

**1.10.V-02 Crude oil hydrocarbon induced soil microbiome shift in polluted soils**

***Chidinma Peace Okafor***[1]*, Chioma Blaise Chikere*[1]*, Onyewuchi Akaranta*[1] *and Khayalethu Ntushelo*[2]*, (1) University of Port Harcourt, Nigeria, (2) University of South Africa*

Crude oil hydrocarbon pollution is a contaminant that alters the environmental quality of affected sites by altering physicochemical parameters of the impacted environment and through the toxicity of the crude oil. This study investigated the effect of crude oil on soil microbiome in a contaminated site in Rivers State Nigeria. The study compared physicochemical parameters, microbial diversity, and functional profiles between the unpolluted portion of the field and the actively polluted soil. It also compared the microbial profile of the polluted soil and after remediation using both chemical (NPK) fertilizer and plant waste respectively using shotgun metagenomics. The polluted soil had total hydrocarbon values greater than 15000mg/kg above the 5000mg/kg regulatory intervention values, a pH of 2.3, and very low nitrogen and phosphate levels. The acidic pH and toxicity of the crude oil resulted in a decrease in microbial population and diversity in the polluted soil when compared with both the unpolluted and treated soils Actively expressed genes within the polluted soil microbiome are the genes for DNA repairs, stress, and chemotaxis, carbon starvation, osmolytes, cryoprotectants and heat shocks, chaperons and hydrocarbon degradation as opposed to a more robust and diverse microbial community with genes and pathways for cell division and nutrient cycling in the unpolluted soil. The remediated sites also had a more diverse microbial population, shifting from the predominant *Proteobacteria* in the polluted soil samples to more diverse microbial phyla including *Verrucomicrobia, Planctomycetes, Actinobacteria,* and *Acidobacteria* in plant waste treatment and *Firmicutes, Actinobacteria* and *Acidobacteria* in NPK treatment. The study demonstrates the importance of soil microbiome in determining contaminant levels and toxicity in the environment.

**1.10.V-03 Antibiotic Resistance Pattern in Hydrocarbon-Degrading Consortium from Crude Oil-Polluted Soil Using Shotgun Sequencing**

***Chioma Blaise Chikere***[1]*, Memory Tekere,*[2] *and Blaise Ositadinma Chikere*[3]*, (1) University of Port Harcourt, Nigeria, (2) University of South Africa, (3) Shell Petroleum Development Company (SPDC), Nigeria*

Bacterial consortium developed from a chronically crude oil-polluted soil was analyzed with ZymoBIOMICS shotgun metagenomic sequencing on an Illumina NovaSeq platform to ascertain the presence of antibiotic resistance genes and virulence factors in the individual species. Axenic cultures were repeatedly screened by enrichment in 1% crude oil- and naphthalene-Bushnell Haas (BH) broth and isolates with enhanced degradation potentials were selected on BH agar. DNA extraction was performed using ZymoBIOMICS-96 MagBead DNA kit while Nextra DNA flex library prep kit was used to prepare sequencing libraries. Raw sequence reads were trimmed to remove low quality fractions and adapters. Antibiotic resistance and virulence factor gene identification were performed with Diamond sequence aligner. Genome analysis by composition barplots at species level in the consortium revealed the presence of *Micrococcus luteus* (83.5%), *M. aleoverae* (14.6%), *M. luteus* Mu201 (0.7%), *M. yunnanensis* (0.3%), *Sphingobium barthaii* (0.1%), *S. fuliginis* ATCC 27551 (0.1%) and *Bacillus persicus* (0.3%). Antibiotic resistance genes affiliated more with *M. luteus* than other species with chloramphenicol efflux MFS transporter Cmx gene having read counts of 950 as the predominant determinant followed by aminoglycoside 6-adenylyltransferase AadK with 2 read counts. Virulence factors such as acetyl-

/propionyl-CoA carboxylase subunit beta and protein disaggregation chaperone, with 253 and 215 read counts respectively were also found only in *M. luteus*. This insight reveals that petroleum degraders in impacted soil microbiome may harbour antibiotic resistance genes and virulence factors of public health concern which need to be considered if such organisms would be used during bioaugmentation as bioremediation agents.

## 1.11.P Poster Only: Environmental Toxicology and Stress Response

### 1.11.P-Tu009 Evaluation of sensitivity of a federally endangered mussel (Tennessee Bean, Venustaconcha trabalis) to selected chemical contaminants

***Ning Wang***[1]*, Chris D. Ivey*[1]*, Danielle Cleveland*[1]*, James Kunz*[1]*, Rebecca Schapansky*[2] *and Timothy Lane*[3]*, (1) U.S. Geological Survey, (2) U.S. National Park Survey, (3) Virginia Department of Wildlife Resources*
Tennessee Bean (*Venustaconcha trabalis*) is a critically endangered mussel endemic to the Tennessee river basin and is on the verge of extirpation throughout its range in Tennessee and Virginia in the United States. Environmental contamination has been identified as a contributing factor to the worldwide decline of freshwater mussel populations. Importantly, previous studies have demonstrated that freshwater mussels are among the most sensitive freshwater species to a variety of contaminants (e.g., ammonia, chloride, potassium, nickel). However, little is known about the sensitivity of the Tennessee Bean. The objective of this study was to evaluate the sensitivity of juvenile Tennessee Bean to 7 chemicals in acute 96-h exposures and to 3 chemicals in chronic 28-d exposures using ASTM standard methods. Test chemicals were selected based on (1) chemicals of potential concern found in a 2021 field survey in the Obed Wild and Scenic River (OWSR), Tennessee, where a population of Tennessee Bean occurs, (2) chemicals to which other mussels are sensitive, or (3) chemicals that had not been previously tested with unionid mussels. A well water diluted with deionized water to a hardness of 100 mg/L as $CaCO_3$ was used as test water. Preliminary results based on nominal exposure concentrations showed that acute EC50s for total ammonia (~6 mg/L as nitrogen), chloride (~1800 mg/L), potassium (~45 mg/L), and nickel (~325 µg/L) were within the range of the EC50s for other mussel species in literature, which indicates that Tennessee Bean has similar sensitivity as other mussels and thus is highly sensitive to these chemicals compared to other freshwater organisms. However, acute EC50s for cobalt (~10 mg/L), manganese (~80 mg/L), and nitrite (~280 mg/L as nitrogen) obtained from our Tennessee Bean tests were relatively high. When comparing to organisms in existing acute toxicity databases, the mussel was ranked among moderately sensitive species to cobalt and among insensitive species to manganese and nitrite. Our chronic toxicity tests with cobalt, iron, and nitrite are ongoing. Results of this study will inform a conservation strategy for recovery of the Tennessee bean, and perhaps other endangered mussel species.

### 1.11.P-Tu010 Respiratory and Cardiovascular Effects Following in vivo Exposure to Respirable Gunshot Residue

***Samuel Cole Smith****, E. Ross Hodges, James A. Stewart Jr. and Courtney Roper, University of Mississippi*
The occupational and recreational use of firearms releases gunshot residue (GSR) into the air and surrounding environment. GSR is comprised of organic and inorganic compounds released after shooting a firearm and includes components with known environmental and human health effects such as Pb, Ba, and black carbon. The particle size of GSR is a key factor in health effects research since particles less than 2.5 microns in aerodynamic diameter, $PM_{2.5}$, are able to enter deep into the respiratory tract and potentially enter into the bloodstream. Currently, there is very limited research investigating the effects of GSR particle size and composition on respiratory and cardiovascular health. This study collected $PM_{2.5}$ during a law enforcement firearms qualification to determine the chemical composition and in vivo effects of exposure. $PM_{2.5}$samples were collected from the firing of 550 rounds of 9mm ammunition during a pistol qualification with shooters wearing air monitors on their upper right arms (n=3). Following collection, the samples were analyzed for black carbon concentrations prior to extraction. Samples and controls (blank filters) were then extracted in 8 mL of methanol and sonicated for 60 minutes to collect $PM_{2.5}$. Samples were pooled together, concentrated, and resuspended in saline. $PM_{2.5}$ samples and controls (vehicle, blank filter) were used to conduct *in vivo* exposures

in wild-type (C57BL/6) mice at 100 µg/mouse. Pre- and 24 h-post exposure physiological parameters were measured using echocardiography to determine cardiac function in the left and right ventricle. Additionally, bronchoalveolar lavage fluid (BALF) was collected to determine total cell counts and cytokine concentrations. An aliquot of the $PM_{2.5}$ solution was analyzed for elements commonly released in GSR including Pb, Ba, and Sb. The average black carbon concentration for the collected $PM_{2.5}$ samples was 1,139 ± 148 $\mu g/m^3$. Mouse exposures and elemental analysis are underway and we anticipate elevated concentrations of heavy metals in the samples and pulmonary hypertension in mice exposed to GSR. We also anticipate that associations between GSR chemical components and physiological measurements will be observed. Ultimately this work will expand the knowledge about potential respiratory and cardiovascular health effects caused by exposure to respirable GSR particles.

**1.11.P-Tu011 Determining the Distribution of Elements and Oxidative Potential across Fine Particulate Matter (PM2.5) Filters**

***Allie Sidwell*** *and Courtney Roper, University of Mississippi*

Fine particulate matter (PM2.5) is a complex mixture of particles and sorbed chemicals that poses serious, adverse human health effects such as increasing cardiovascular and respiratory morbidity and mortality. There is ongoing research into the impacts of PM2.5 with differing chemical compositions and sampling locations, as well as work to identify the mechanisms for the observed health effects. To conduct these analytical and toxicology studies of PM2.5, researchers often split filters into sections. This process allows multiple, often destructive, assays to be performed. Our preliminary research showed chemical composition differences across PM2.5 filters. The goal of our study was to determine the validity of splitting filters for use in multiple analyses, analyze differences between an urban and rural sampling location, and examine trends between PM2.5 components and toxicology by assessing differences in chemical composition and oxidative potential within the same filter. PM2.5 filter samples collected from an urban and a rural location were used (n=3 filters/location). Each filter was split into quadrants, resulting in a total of 24 pieces; laboratory and blank filters were also prepared in the same manner. Each filter piece was extracted, concentrated, and then analyzed with the dithiothreitol (DTT) assay run in triplicate to determine oxidative potential. Inductively coupled plasma mass spectrometry (ICP-MS) was run on all samples and controls to compare chemical composition of the filter quadrants (n=30). Stark differences in total elemental content and oxidative potential, up to an eight times difference, were observed between quadrants of the same filter. Correlation analysis between oxidative potential and elements yielded significant, positive correlations between oxidative potential normalized by PM2.5 mass and the elements Ag, Ba, Cr, and Ga at the rural location. At the urban location, a significant, negative correlation between oxidative potential normalized by PM2.5 mass and the element Cs was observed. This work will provide information about the feasibility of splitting PM2.5 filters for multiple analyses on the same sample as well as insight into the different sources and toxicology of PM2.5 components.

**1.11.P-Tu013 Diet Effects Egg Laying, Biomass, and Stable Isotope Values in Tetragnathid Spiders: Use of a Novel Laboratory Technique**

***Sarah Kerr*** *and Ryan R Otter, Middle Tennessee State University*

Spiders within the family Tetragnathidae are used by researchers to study the linkage between aquatic and terrestrial food webs, including the movement of contaminants across these two ecosystems. Using a novel laboratory protocol (14-day duration), we investigated the effects of dietary consumption (house flies) on female spiders by creating different feeding groups and daily measuring adult mass, egg laying frequency, egg sac mass, and total number of egg sacs laid. We also evaluated the effects of dietary consumption and dietary source on the isotopic signatures (carbon and nitrogen) of spiders in two different tissues (body & legs). Results revealed that adult mass was not dependent on consumption amounts, however spiders that consumed more flies had greater egg sac masses, egg laying frequency, and total egg sacs laid. Dietary consumption amounts and diet source did not affect nitrogen isotope signatures but did affect the carbon isotope signatures in individual spider bodies, but not legs, showing that carbon turnover rates differ based on tissue type.

**1.11.P-Tu014 Metabolic Profiling of Daphnia magna Exposure to Phthalates Using Targeted Liquid Chromatography Tandem Mass Spectrometry**

***Erico Oliveira Pereira***[1]*, Lisa Labine*[1]*, Sonya Kleywegt*[2]*, Karl Jobst*[3]*, Andre Simpson*[1] *and Myrna Simpson*[1]*, (1) University of Toronto, Canada, (2) Ontario Ministry of the Environment, Conservation and Parks, Canada, (3) Memorial University of Newfoundland, Canada*

Phthalic acid esters, also known as phthalates, are commonly used chemicals in the production of personal care and plastic products. As a result of their multitude of applications, phthalates are widely detected in aquatic ecosystems. Some phthalates can be biodegraded, which results in the transformation of phthalate diesters into monoesters. Their widespread presence has been reported to impact the health of freshwater organisms. *Daphnia magna* is a small crustacean frequently used in ecotoxicology as a sentinel species for aquatic toxicity studies. Toxicological endpoints of *D. magna* have been investigated for several phthalate pollutants but more information about how they disrupt the sentinel species' health is needed. The current study compared the *D. magna* metabolic profiles of two phthalate diesters (dimethyl phthalate and diethyl phthalate) and two phthalate monoesters (monomethyl phthalate and monoethyl phthalate) to better understand their short-term toxicity. In a 48-h test, *D. magna* was exposed to sub-lethal concentrations of all four phthalates. These concentrations were fractions of the $EC_{50}$ values (pollutant concentration that induces immobilization in half of the population) for each phthalate. Targeted analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to measure 51 polar metabolites from which 46 were detected. Multivariate analyses were used to investigate changes in metabolite profiles of different exposure groups relative to the control (unexposed group). Partial least squares-discriminant analysis (PLS-DA) showed separation from the control group for all phthalates, indicating metabolic perturbations for all pollutants. Metabolite percent changes and pathway analysis were used to investigate disruptions to biochemical pathways. Across the four phthalate pollutants, pathways associated with protein synthesis and amino acid metabolism were disrupted. In addition, each phthalate had unique metabolite changes, which suggests some specificity in their toxicity. The metabolic profiling of *D. magna* indicated that not all phthalates impacted the species in the same manner, however, associated biochemical pathways were disturbed, signalling a general chemical class response. These results demonstrate that phthalate pollutants invoke similar and unique responses in aquatic organisms that cannot be directly correlated to other commonly assessed endpoints.

**1.11.P-Tu015 Does Venlafaxine Alter Metabolism of Zebrafish via Mitochondrial microRNAs?**

***Karyn Robichaud*** *and Paul Craig, University of Waterloo, Canada*

Wastewater effluent that is released into freshwater ecosystems can have a variety of negative effects on fish health. One contaminant that is often found downstream of wastewater treatment plants is venlafaxine (VFX)- an anti-depressant drug that causes behavioral and metabolic effects in zebrafish. However, the mechanisms regulating how VFX elicits these effects are not fully understood. We hypothesized that microRNA, small RNA molecules that post-transcriptionally regulate gene expression, are transported into the mitochondria where they regulate expression of mitochondrial genes, namely oxidative phosphorylation protein subunits, resulting in a change in metabolism. We previously demonstrated that microRNA are present in the mitochondria of three species of darters, a benthic freshwater fish, and predicted that they play a role in silencing mitochondrial protein expression. In this study, it was hypothesized that VFX alters mitochondrial respiration in zebrafish, and that this change in metabolism is in part caused by microRNA silencing of mitochondrial genes. First, zebrafish were exposed to treatments of VFX to validate the effect of VFX exposure on mitochondrial respiration. Following exposures, mitochondrial fractions of target tissues were isolated, candidate microRNA and their target mRNAs were quantified using qPCR, and cytochrome c oxidase activity was measured. This novel research described how mitochondrial microRNAs impact mitochondrial gene expression in response to venlafaxine exposure in zebrafish and will contribute to scientific understanding of how microRNA regulate mitochondrial gene expression in fish in response to stress overall.

**1.11.P-Tu016 Genetic Signatures of Selection for Resistance to Lead Exposure in the Turkey Vulture (Cathartes aura) Genome**

***Alexandria Koedel***[1], *Andrea Bonisoli Alquati*[1], *Alexandra Gresham*[1], *Peter Bloom*[2], *Miguel Saggese*[3] *and Allison Shultz*[4], *(1) California State Polytechnic University, Pomona, (2) Bloom Research Inc., Malta, (3) Western University of Health Sciences, (4)Natural History Museum of Los Angeles County*

Turkey Vultures (*Cathartes aura*), key scavengers in many ecological communities', often feed on carcasses left behind by hunters who use lead (Pb) shot, resulting in their exposure to this toxic metal and a potential population genetic response. Currently, no information exists about genetic variation in susceptibility to Pb in vultures or whether past exposure to Pb has exerted selection for increased resistance to Pb in this species or any other raptor. Signatures of selection may be identified in the genome through detection of the "hitchhiking effect", in which selectively favorable mutations are fixed in a population thus altering the frequency of alleles at closely linked loci. Consistent with this, we expect signatures of selection at loci associated with Pb detoxification or sensitivity, as well as other changes such as structural variants and rearrangements. We are currently assembling the genome using reads from Illumina short-read sequencing of 10 Turkey Vultures and a previous high-quality Turkey Vulture reference genome. Through genomic analysis methods, we will identify signatures of selection in regions of the Turkey Vulture genome using outlier loci tests and neutrality test statistics such as Fay and Wu's and Tajima's D test. By running tests like those forementioned, we will be able to additionally distinguish between positive selection occurring since divergence from common ancestors and recent selection. Evidence of genetic signatures of selection for resistance to Pb exposure in the Turkey Vulture genome will unveil how raptor populations might face toxicological risk from Pb pollution, and what the role is for evolution (as opposed to plasticity), in determining sensitivity to Pb contamination.

**1.11.P-Tu017 Early developmental exposure to Δ9-tetrahydrocannabinol causes persistent multigenerational hyperactivity and altered brain mitochondrial function in zebrafish**

***Zacharias P Pandelides***, *Morgan Appenrott, Victoria L. Jackson, Cammi Thornton and Kristine L. Willett, University of Mississippi*

A major constituent of cannabis, Δ9-tetrahydrocannabinol (THC), is a contaminant of emerging concern due to limited information on its environmental impact. Due to THCs known modes of action, evaluation of the potential adverse neurological effects following exposure to THC during early development is crucial. Following exposure to 0.08, 0.4, or 1 µM THC from 6-96 hours post fertilization (hpf) in wild-type (5D) zebrafish, latent behavioral effects caused by THC were assessed at 120 hpf (larval), 3 weeks post fertilization (wpf) (juvenile), 11 wpf (onset of sexual maturity) and 24 wpf (adult). Using the larval photomotor response test (LPR), hyperactivity in the dark phase was evident in fish exposed to 0.4 µM THC. Open field tests (OFT) conducted at 3 and 11 wpf indicated dose-dependent hyperactivity and increased thigmotaxis at the two highest THC concentrations. Effects on thigmotaxis did not persist into adulthood. Yet, adult zebrafish behavior in the OFT revealed significant hyperactivity (increased velocity) amongst both sexes of fish exposed to 0.4 or 1 µM THC. Furthermore, hyperactivity in the 120 hpf LPR test persisted into the F1 generation, offspring of the developmentally exposed fish at all concentrations tested. Developmental exposure to THC also caused significant changes to the size of the F0 fish by 24 wpf, with significantly lower and higher weights in fish exposed to the lower and higher THC concentrations, respectively. Developmental exposure to THC also caused persistent alterations in brain mitochondrial function such as increased basal oxygen consumption rate and reserve capacity. The role of cannabinoid receptor 1 (CB1) in mediating THCs effects on behavior was also measured using *cnr1*$^{-/-}$ zebrafish, which suggested that the anxiety-like behavior was mediated by CB1 but hyperactivity was not. Collectively, these results showed that exposure to THC during a critical period of development caused behavioral and mitochondrial alterations that persisted into adulthood and the F1 generation. This work was supported by the National Institute on Drug Abuse R21DA044473-01.

**1.11.P-Tu018 Effects of 17α-ethinylestradiol (EE2) on Gonadal Development and Gene Expression in Larval Mummichog (Fundulus heteroclitus)**

***Carly Barbara Anna Sing-Judge***[1], *Glen Van Der Kraak*[2] *and Deborah MacLatchy*[3], *(1) University of Waterloo, Canada, (2)University of Guelph, Canada, (3)Wilfrid Laurier University, Canada*
The mummichog (*Fundulus heteroclitus*) is a small-bodied estuarine killifish that inhabits the North American east coast and is often used as a model adult saltwater fish in environmental bioassays. This study describes gonadal development and gene expression levels in five-week-old mummichog following 17α-ethinylestradiol ($EE_2$) treatment after hatching to better understand the susceptibility of sex determination (SD) and gonadal differentiation (GD) processes following contaminant exposure. Harvested eggs were fertilized, collected, and incubated for three weeks until the time of hatching. Yolk-sac larvae were reared for five weeks in one of three static (daily renewal) treatments [0, 2 and 10 ng/L $EE_2$ (actual)] and sampled for gonadal histology and gene expression analysis. The sex ratio approached 50/50 in controls and $EE_2$ treatment did not alter this ratio. There was no evidence of intersex gonads. Several genes implicated in GD in fish were identified for the first time in the mummichog including *cyp19a1a*, *foxl2*, *gdf9*, *bmp15*, *dmrt1*, and *amh.* $EE_2$ treatment did not change ovarian gene expression, whereas testis expression was significantly decreased in *dmrt1* and increased in *cyp19a1a*, *foxl2*, *gdf9*, and *amh*. Hindered gametogenesis and increased degeneration were observed in $EE_2$-treated ovaries and testes, indicating that GD mechanisms were sensitive to $EE_2$ treatment after hatching. The lack of effect of $EE_2$ on sex ratio was surprising and may have been a result of treating the fish after hatching and not during embryogenesis. These results demonstrate that male mummichog are more sensitive to $EE_2$ after hatching than females. This study suggests that SD in the mummichog is fixed prior to hatching and that GD mechanisms are susceptible to environmental estrogen exposure during early development.

### 1.11.P-Tu019 Ancestral BPA Exposure Led to Follicular Atresia and Metabolic Diseases in the 5th Generation Grandchildren

***Sourav Chakraborty***, *University of North Carolina Greensboro*
BPA, a ubiquitous chemical contaminant, can cause reproductive impairment in fish and other vertebrates. Direct BPA exposure can disrupt oocyte differentiation and cause follicular atresia in the ovary. However, it is unclear if ancestral BPA exposure can lead to transgenerational disruption of ovarian function several generations after a BPA exposure. Here we show follicular atresia in the ovary of the F4 generation (five generations after exposure to BPA) females, which was accompanied by hyperactivity of vitellogenin and estrogen receptor alpha gene in the liver. The ovary of Medaka (*Oryzias latipes) fish* whose great grandparents were exposed to an environmental concentration of BPA (10 g/L) during their first 12 days of embryonic life was examined. A disrupted progression of cortical alveolar stage II oocyte to early vitellogenic stage III was found in the females from ancestrally BPA exposed lineage. The follicular stage and follicular areas were quantified by ImageJ. Morphometric analysis of follicular stage showed a significantly higher number of previtellogenic oocytes with big atretic follicles in the fish from BPA lineage. Expression of lhr gene, which is associated with a post-vitellogenic state (*lhr*), was downregulated and conversely, the vitellogenin receptor gene (*vldlr*), testosterone synthesizing gene (*3b-hsd*), and apoptotic marker gene (*casp3*) were unregulated, suggesting transgenerational indirect BPA exposure effects on oocyte maturation and degeneration. In addition, BPA lineage fish developed transgenerational nonalcoholic fatty liver disease (NAFLD). The liver transcriptome revealed a significant increase in the expression of *vitellogenin* and *esr* genes due to ancestral BPA exposure, which implies abnormal crosstalk in the liver-ovary axis. The present results suggest that BPA induces transgenerational reproductive and metabolic disease phenotypes in offspring after several generations of exposure.

### 1.11.P-Tu020 Transcriptional Alterations Induced by Potassium Perchlorate Exposure in the Adult Medaka Testis

***Beh Reh***, *Yashi Feng and Ramji K. Bhandari, University of North Carolina Greensboro*
Perchlorate is a chemical compound commonly used in military artillery and equipment. It has been detected in drinking water, air, soil, and breast milk. Human exposure can occur in the theatre of war and areas adjacent to military training grounds. A high concentration of perchlorate has been found to affect reproduction in

vertebrates including fish. However, whether environmental concentrations of perchlorate can affect male reproduction is not clearly understood. This study examined DNA methylation profile of the sperm and transcriptional alteartions affected by potassium perchlorate in the testis of adult medaka. Male medaka were exposed to 0, 10, and 10000 ug/L potassium perchlorate and 10000 ug/L perchlorate+3 mg/L vitamin C for 21 days. Vitamin C was used together with perchlorate to see if epigenetic effects of perchlorate can be amerliorated by vitamin C as vitamin C serves as a global genome demethylating agent in *vivo*. Perchlorate exposure caused reduced fertility in males and altered survival rates of the F1 offspring. Sperm genome methylation data is currently under analysis. Transcriptome profiling of the perchlorate exposed testis showed significant alterations in focal adhesion, nuclear integrity, and spindle formation, and phospholipid binding in the testis of both low and high concentration perchlorate exposed individuals. Co-treatment of fish with high concentration perchlorate and vitamin C resulted in an increased expression of several genes representing pathways involved in Rap1 signaling, calcium signaling, non-coding RNA processing, spindle formation, and cell substrate junction. Results suggest that vitamin C can induce transcriptional network involved in the protection of testicular functions against perchlorate exposure. [*Funded by STAMPS program UNC Greensboro, URSCO Summer Research Award, Department of Biology Undergraduate Research Fund, UNC Greensboro Honors College to BR*]

**1.11.P-Tu021 The Florida Red Tide Toxin Induces a Massive Shift in the Redox Proteome of Lymphoblast Cells Which Can Be Reversed With the Acrolein Scavenger MESNA**
***Kathleen Rein***, *Jordan Jobson, Eman Taher, Pawlos Tsegay and Yuan Liu, Florida International University*
*Karenia brevis* is a marine dinoflagellate that is endemic to the Gulf of Mexico. This species is responsible for the Florida red tide blooms that occur almost annually. *K. brevis* produces brevetoxins, which induce various neurological symptoms in humans upon consumption of tainted fish or shellfish, as well as respiratory distress, particularly in asthmatics, upon inhalational exposure when toxins are aerosolized in sea spray. Manatees, dolphins, sea turtles, and sea birds are just a few examples of marine life that experience mass mortality events and strandings due to this harmful algal bloom. Florida red tide blooms result in significant economic losses to the Gulf States, particularly the tourism and aquaculture industries. Brevetoxins bind to and activate voltage-gated sodium channels lowering the activation threshold and prolonging open times resulting in neurological symptoms. In addition, we have recently discovered that the most abundant of the brevetoxins (PbTx-2) is an inhibitor of mammalian (rat) and *Karenia brevis* thioredoxin reductase (TrxR) a major regulator of cellular redox status. Model organisms and cells exposed to brevetoxins in laboratory experiments and animals rescued from red tide show enhanced indicators of oxidative stress which may be consequence of TrxR inhibition. To assess the effect of PbTx-2 on the proteme, we have performed redox proteomics on PbTx-2 treated lymphoblast cells. These cells show a massive shift in the status of reversible cysteine oxidation compared to control (untreated) cells. We have also determined that the redox status of the proteome can be restored to control levels by simultaneous treatment with PbTx-2 and sodium mercaptoethyl sulfonic acid (MESNA). MESNA may function as an antioxidant or as a brevetoxin scavenger. Additionally, we have cloned and expressed human thioredoxin reductase-1 (hTrxR-1) to evaluate the effect of PbTx-2 on this enzyme.

**1.11.P-Tu022 Synthesis of mercaptan-base drugs and pharmacological evaluation against brevetoxin toxicity on voltage gated sodium channel and thioredoxin system**
***Mayra Alejandra Tabares Beltran*** *and Kathleen Rein, Florida International University*
Brevetoxins (PbTx), neurotoxins produced by *K. brevis* are believed to be responsible for marine mammal mortalities, poisonings, and deaths of fish, sea birds, turtles, as well as adverse human health impacts during red tide events. Studies have identified two different modes of action executed by these marine toxins: binding to the voltage-gated sodium channel (VGSC) or inhibition of thioredoxin reductase (TrxR), a major regulator of cellular redox homeostasis. Nowadays, there are no current treatments available for the neurotoxic or respiratory effects produced by brevetoxin either on wildlife or humans. Therefore, we propose the development of “antitoxins” based on two FDA-approved acrolein scavengers; (1) sodium 2-mercaptoethane sulfonate

(MESNA), and (2) cysteamine, which will effectively detoxify PbTx-2, the most abundant of the brevetoxins. MESNA is co-administered with ifosfamide and cyclophosphamide to target the metabolic by-product, acrolein and cysteamine is used in the cases of cystinosis and is related with induction of glutathione concentration during acetaminophen overdose. Enhancement of their physicochemical properties is required due to their high hydrophilicity and short half-life lives. In this study, developed in four steps, we will perform: (1) synthesis of sulfonamide, sulfonate ester and amide derivatives of MESNA and cysteamine with enhanced lipophilic properties. (2) Analysis of their chemical properties and reactivity with PbTx-2 will be conducted by determining the octanol-water partition coefficient and assessing their ability to form PbTx adducts by Michael reaction. (3) Voltage-gated sodium channel binding and (4) inhibition of the human thioredoxin reductase 1 (hTrxR-1) by PbTx adducts. We hypothesize that this new mercaptan-based drugs will form conjugates with PbTx-2 and decrease its cytotoxic effects by three mechanisms: affecting the binding affinity with the site 5 on the voltage-gated sodium channel, preventing the inhibition of the thioredoxin system, and by alleviating the oxidative stress due to their antioxidant properties. Further results will provide valuable information as an emerging alternative treatment for brevetoxicosis in wildlife or humans.

**1.11.P-Tu023 An Integrated Systems-Level Model of Zearalenone Toxicity in Fish Embryos Based on NMR Metabolic Profiling**

***Mark Annunziato**, Florida International University*

Zearalenone (ZEA) is a mycotoxin that has been detected worldwide in an array of cereal crops typically being of concern to humans and land-based livestock. Recently, ZEA has also been shown to enter the aquatic environment via runoff from agricultural fields and occur in commercial feed. Despite this, there remains a lack of uniform understanding about the toxicological impact of ZEA on fish species. To uncover a systems-level understanding of toxicity of Zearalenone, high-resolution magic angle spinning nuclear magnetic resonance (HRMAS NMR) was utilized for metabolic profiling of embryonic stages of zebrafish (Danio rerio) and olive flounder (Paralichthys olivaceus). Through HRMAS NMR metabolic profiling of intact fish embryos, more than 30 metabolites were identified with over a third of them significantly altered in ZEA exposed fish, allowing for a systems-level model of ZEA toxicity to be constructed.

**1.11.P-Tu024 Using Zebrafish Behavior To Reveal Gene-Environment Interactions Underlying Population Susceptibility Differences In Response To Environmental Chemical Stressors**

***Preethi Thunga**[1], Lisa Truong[2], Yvonne Rericha[2], Warren Anderson[2], Arianna Sanchez[2], Jane La Du[2], Russell Schaaf[2], Mackenzie Moorehead[2], Robyn Leigh Tanguay[2] and David Reif[1], (1) North Carolina State University, (2) Oregon State University*

Chemical exposures have been linked to numerous adverse health effects. However, in genetically diverse populations, many exposures display broad susceptibility differences across individuals. Thus, understanding the role of gene-environment interactions (GxE) in differential susceptibility to an expanding chemical exposome is key to protecting public health— particularly that of vulnerable populations. Moreover, while screening emerging contaminants such as Per- and Polyfluoroalkyl Substance (PFAS) for toxicity, it is important that we flag chemicals that can induce such differences in disease susceptibility. Our group and others have previously shown that larval zebrafish (*Danio rerio*) behavior is highly useful for high-throughput toxicity screening. For certain chemicals, these larvae show considerable levels of inter-individual variation in their behavioral responses. Quantifying effects of underlying genetics on the observed behavioral plasticity will allow us to answer questions regarding the implications of GxE and identify signals related to differential susceptibility. We used a family-based design to estimate heritability (amount of phenotypic variation in a trait that can be attributed to genetic variation) from a larval behavioral assay of zebrafish raised in a chemical-free environment versus those exposed to the PFAS, Perfluorohexanesulfonic acid (PFHxA). We found that heritability of the PFHxA exposed populations was 20.5%, versus only 4.8% for control populations. Furthermore, the phenotypic variance amongst pairs of PFHxA-treated fish differed significantly (Levene's test p-value $<< 0.05$), but this same effect was not seen amongst the different groups of unexposed control fish. To probe whether such differences in behavioral phenotypes can be attributed to underlying differences in RNA expression, we also estimated transcriptomic heritability. This study shows evidence that heritable genetics can affect behavioral susceptibility of individuals to PFHxA exposure and that the standing genetic variation in our population can be harnessed to reveal GxE underlying population susceptibility differences.

### 1.11.P-Tu025 Investigating the Impacts of Irbesartan on Human and Zebrafish PPARγ via In vitro Bioassay Testing and In vivo Zebrafish Models

***Nima Madani***[1]*, Maira Oliva*[1]*, Jake Ulrich*[2]*, Joseph H. Bisesi Jr.*[1]*, Nishad Jayasundara*[2]*, Lee Ferguson*[2] *and Tara Sabo-Attwood*[1]*, (1) University of Florida, (2) Duke University*

Lack of knowledge about the presence of chemicals in diverse water systems makes prioritization of contaminants of concern to human and ecosystem health a significant challenge. The toxicity associated with numerous suspect chemicals is available in open-source databases, such as ToxCast, but these databases are primarily focused on those chemicals that have been identified in environmental samples heavily focusing on agricultural compounds (i.e., agrochemicals). In efforts to increase our identification chemical identification capacity, we are employing 'non-targeted' high-resolution mass spectrometry (NTMS) to generate chemical fingerprints in water samples to determine whether toxicity data are available for these chemicals in ToxCast. For understudied chemicals, we are then performing *in vitro* and *in vivo* toxicity testing using a zebrafish model. For this study we collect water samples and chemical profiles are generated by LC-MS/MS from Haiti, North Carolina, and Sri Lanka. In total, 169, 144, and 323 unique chemicals are identified in Haiti, Sri Lanka, and North Carolina respectively with 11 shared by all regions. ToxCast is then mined for each chemical. Results of this analysis show that 60% of all compounds are not represented. For those that are identified, it is noted that pharmaceuticals have lower representation (49%) compared to pesticides (84%). We identify irbesartan, a pharmaceutical used to control blood pressure, as a candidate for further investigation by using a prioritization scale based on limited knowledge of toxicity in ToxCast and the literature, and because it was found in all water samples across the three different regions. Irbesartan can activate PPARγ in mammalian systems, but limited information is available for aquatic species. To begin to understand PPARγ-mediated toxicity, we employed transactivation reporter assays for human and zebrafish PPARγ. Irbesartan was able to activate both PPARγ receptors, resulting in AC50 values of 11 μM and 13 μM for human and zebrafish, respectively. In preliminary studies, zebrafish embryos were exposed to 3 doses of irbesartan (1, 5, and 10 mg/L) resulting in significant behavioral changes only at the lower doses. Future measures will evaluate changes in adipogenesis, energy metabolism, heart rate, and lipidomics. These efforts should lead to a better understanding of exposure and health effects of irbesartan and other similar pharmaceuticals to aquatic species.

### 1.11.P-Tu027 Redox status of blood tissue associated with the ingestion of arsenic from groundwater and methylation efficiency in populations of the Colombian Caribbean

***Farith Damian Gonzalez***[1]*, Jessica Mercado-Páez*[1]*, Boris Johnson-Restrepo*[1]*, Luis Quiñones*[2]*, Ramon Rodrigo*[2] *and Darío Mendez*[1]*, (1) University of Cartagena, Colombia, (2) University of Chile*

Inorganic arsenic (InAs) is considered to be the principal form of arsenic (As) in groundwater. USEPA guideline for As in water is 10 μg/L. Exposure to As leads to increased production of reactive oxygen species (ROS) and other toxic intermediates through its biotransformation, which can subsequently cause oxidative stress. However, these effects can vary largely among individuals, possibly due to demographic, anthropometric factors, smoking history, lifestyle and genetic factors, increasing the susceptibility to As toxicity. This study evaluated the redox status in the blood tissue of people exposed to As by drinking groundwater and their possible association with methylation efficiency. Specific surveys on 155 individuals aged between 18 and 81 years old were applied used to collect demographic and anthropometric information of the subjects and their lifestyle. The groundwater and urine samples were analyzed for assessing using HPLC-HG-AFS. Likewise, the exposure and risk of As was assessed by Lifetime average daily dose method (LADD) and the Hazard quotient (HQ). The methylation efficiency was determined for urinary speciation. Besides, the redox status of blood tissue was determined by oxidative stress tests such as Superoxide dismutase (SOD), Catalase (CAT) and

Glutathione (GSH). In addition, the Carbonyl Index (CI) and Ferric Reducing Antioxidant Power (FRAP) were used. The association among LADD and the redox status in blood tissue were tested using a multivariate analysis, adjusted by potential confounders. Sixteen groundwater wells from studied municipalities were analyzed. The study population was divided post hoc into two subgroups: a subgroup exposed to As above optimal levels (n=55), with aquifer concentrations of 16 µg/L, and a subgroup exposed to As below optimal levels, with aquifer concentrations of 7.2 µg/L (n=100). The LADD in the subgroup with high exposure was of 0.31 µg/kg-bw/day, generating a risk HQ=1.1. The urinary arsenic species concentrations were 0.80 µg/L for InAs, 0.60 µg/L for $MMA^{V}$ and 1.2 µg/L for $DMA^{V}$. The values of SOD and CAT were higher in individuals from the subgroup with high As exposure (p= 0.03 and p= 0.04 respectively). Besides, were positively correlated with urinary As concentrations. Exposure to inorganic As was associated with increased redox status among individuals of the Colombian Caribbean. Therefore, SOD, CAT and FRAP could serve as biomarkers for assessing the effects of As in humans.

**1.11.P-Tu030 Seasonal Variation in Quagga Mussel Cellular Biomarkers and DNA Damage in the Great Lakes: Implications for Bivalve Environmental Biomonitoring**

***Neil Fuller***[1]*, Ashley Elgin*[2]*, Kimani Kimbrough*[2]*, Ed Johnson*[2]*, Erik Davenport*[2]*, Pawel Jaruga*[3]*, Annie Jacob*[1] *and Amy Ringwood*[4]*, (1) CSS Inc./NOAA, (2) National Oceanic and Atmospheric Administration (NOAA), (3) National Institute of Standards and Technology (NIST), (4) University of North Carolina at Charlotte*

Biomarkers including acetylcholinesterase (AChE), glutathione (GSH), lipid peroxidation (LPx) and DNA damage have been widely used as indicators of chemical exposure and effects in bivalves in the laboratory and natural environment. However, previous studies have demonstrated that environmental and physiological parameters including temperature and reproductive condition may influence biomarker response in bivalves. Consequently, understanding seasonal changes in bivalve biomarkers is fundamental to disentangling the effects of contaminants and other abiotic and biotic factors in the natural environment. Previous studies have demonstrated seasonal differences in biomarkers of mytilid and zebra mussels, however this has not been determined for quagga mussels (*Dreissena rostriformis bugensis)*, an emerging model for environmental biomonitoring in the Great Lakes region. To address this knowledge gap, quagga mussels were repeatedly collected from the mouth of the Muskegon River (Lake Michigan), a site of known low contamination relative to other areas within the lakes, and monitored for levels of AChE, total GSH, lipid peroxidation and seven oxidative DNA damage markers from May – November 2018. In conjunction with mussel collections, environmental conditions including temperature, dissolved oxygen, cyanobacteria pigment and chlorophyll a were monitored, coupled with analysis of mussel ash-free dry weight (AFDW), condition index and gonad fullness. Among the cellular biomarkers, levels of AChE appeared most related to water temperature, with maximum values observed at the two temperature extremes in August and November. Total GSH was not related to environmental conditions but appeared most related to the reproductive cycle, with highest levels of GSH coinciding with peak gonad fullness, followed by a significant decline post-spawning. LPx exhibited few temporal trends, but appeared to be inversely related to water temperature. Levels of several oxidative DNA damage markers, including 5-OH-Me-Hyd and Rcd-Guo, were induced at higher temperatures, whilst 5-OH-Ura appeared to be related to a spike in pesticide levels in mussel tissue. These findings highlight the importance of considering both environmental and physiological conditions in environmental biomonitoring studies with bivalves, and provides baseline information that will underpin future use of quagga mussels as a model species in bivalve health monitoring.

**1.11.P-Tu031 Determining the Role of Fungicides in the Development of Antibiotic Resistant Bacteria.**

***Natalie Wieber****, Paul Koch and Jeri Barak, University of Wisconsin, Madison*

Antibiotic resistant bacteria cause 2.8 million infections and more than 35,000 deaths in the United States annually. Studies have shown that the application of fungicides can cause an increase in the abundance of bacterial genes responsible for antibiotic resistance within soil, however, the same has not been shown on plants. To investigate this relationship, we are applying fungicides with various modes of action to plants over the course of a growing season, sampling bacteria on the surface of the leaf, and measuring the abundance of antibiotic resistance genes through shotgun metagenomics. We hypothesize that fungicides will increase the abundance of antibiotic resistance genes on plants with broad-spectrum fungicides and mixtures of fungicides causing the greatest increase. This investigation will provide further insight into factors that increase the abundance of antibiotic resistance genes which can serve as a reservoir for clinically relevant antimicrobial resistant bacteria.

**1.11.P-Tu032 Investigation of Toxicity of Selected Fluorinated Pharmaceuticals via In Vitro Bioluminescence Assay Using Allivibrio fisheri**

*Heegu Kim and* ***Jong Kwon Choe****, Seoul National University, Korea, Republic of (South)*

Recently, fluorinated pharmaceuticals have been synthesized and replacing their non-fluorinated analogues in the pharmaceutical industry. Substitution of C-H with C-F bonds in fluorinated pharmaceuticals resulted in enhancement of pharmacological effects in the human body, mainly due to improved lipophilicity and metabolic stability. Currently, over 20% of the drugs in the market are fluorinated pharmaceuticals. While their occurrence in aquatic environment have been previously reported, studies on their toxicological effects to living organisms in the environment are still limited. The current study aims to investigate whether fluorinated pharmaceuticals pose more threat to human and natural environment than other pharmaceuticals. Fifteen fluorinated pharmaceuticals (celecoxib, emtricitabine, ezetimibe, fluconazole, fluticasone propionate, lansoprazole, leflunomide, voriconazole, atorvastatin, enrofloxacin, fluoxetine, levofloxacin, risperidone, rosuvastatin, sitagliptin) were chosen, and Microtox assay with the marine bacterium *Allivibrio fischeri* was used as an *in vitro* short-term screening assay to assess the cytotoxicity of these compounds. Results were compared with those of other pharmaceutical and environmental chemicals using a general baseline toxicity Quantitative Structure-Activity Relationship (QSAR) using liposome-water distribution ratios as descriptors. Additionally, toxicity of selected fluorinated pharmaceuticals (fluoxetine, emtricitabine, lansoprazole) and their non-fluorinated analogues (atomoxetine, lamivudine, rabeprazole) were directly compared to investigate the influence of fluorinated moieties. Results from the current study will provide preliminary basis to determine whether fluorinated pharmaceuticals need more attention on their occurrence and fate in the natural and engineered environmental systems.

**1.11.P-Tu033 Zebrafish High-Throughput Assay for the Evaluation of Pesticide-Specific Mitochondrial Toxicity, Survival and Deformity Outcomes**

***Melissa Chernick***[1]*, Lindsay Jasperse*[1]*, Prabha Ranasinghe*[1]*, Jacob Smoot*[1]*, Nicholas Geitner*[1,2]*, Nishad Jayasundara*[1] *and Richard Di Giulio*[1]*, (1) Duke University, (2) Syngenta Crop Protection*

Chronic ecotoxicity studies are time consuming and expensive, particularly with vertebrates. Early life stage fish models used in combination with high-throughput sub-organismal assays can allow for the rapid assessment of many chemicals. Severe developmental deformities generally lead to mortality early in life while sub-organismal alterations can impact the long-term health of the organism. Mitochondrial dysfunction is an example of a physiological (or sub-organismal) alteration that affects critical cellular function and potentially overall fitness. This study focused on two sets of non-lethal endpoints to assess and predict chronic toxicity outcomes following exposure to various agrochemicals. Agrochemical compounds fell into three categories: fungicides, insecticides, and herbicides. Zebrafish (*Danio rerio*) embryos were exposed to various compounds in a range of concentrations starting at 6 hours post-fertilization (hpf). Individuals were assessed daily for survival and evaluated for developmental alterations at 96 hpf. Next, embryo mitochondrial function was measured across a sub-teratogenic range of concentrations for each compound using an Agilent 96-well Seahorse Extracellular Flux Analyzer. This technology allows for the targeted measurement of components of mitochondrial respiration within a live embryo. Results showed a wide range of phenotypic changes, with many compounds having non-specific patterns of deformities while others had compound-specific alterations. For some compounds, mitochondrial assays identified portions of the respiration cycle affected by exposure even at concentrations well below those that resulted in developmental changes. For the majority of compounds evaluated, however, these analyses indicated that developmental deformity assays may be a more sensitive endpoint compared to the mitochondrial assay. Finally, a small proportion of the compounds resulted in no changes for any of the endpoints tested. The zebrafish embryo proved to be a rapid, inexpensive vertebrate model that can be used for safety evaluations, environmental monitoring, screening of novel substances, and predictive modeling of developmental toxicity.

**1.11.P-Tu034 Developmental effects of in ovo exposure to six polycyclic aromatic hydrocarbons in chick embryos**

***Yulianis Pagan-Agosto****, Hallum Ewbank and Christopher G. Goodchild, University of Central Oklahoma*

Following oil spills, avian embryos may be exposed to polycyclic aromatic hydrocarbons (PAHs) when crude oil is transferred from oiled nesting material or oiled feathers of brooding parents to the eggshell surface. While several studies have examined the effects of PAHs on adult birds, the developmental effects of embryonic exposure to PAHs remain unclear. In other taxa, like fish, embryonic exposure to PAHs causes cardiac impairments like bradycardia and a decline in cardiac output. Similar trends have been detected in avian embryos, specifically external application of crude oil to the eggshell reduces embryonic heart and metabolic rates. However, the sublethal toxic effects and the specific PAHs driving these effects in developing avian embryos are still poorly understood. This experiment investigated the effects of sublethal exposure of six PAHs (anthracene, phenanthrene, pyrene, chrysene, benzo[a]pyrene, and fluoranthene), at four different concentrations (100, 200, 400, and 800 ng PAH / g egg mass), on avian embryonic heart rate, organ mass, and morphology. We exposed chicken embryos to PAHs on embryonic day (ED) 3 via egg-injection. We recorded heart rate on ED 10, 14, and 18, and collected organ mass and morphology data on ED 18. We saw a decrease in ED 18 heart rate at the highest concentrations for fluoranthene, phenanthrene, chrysene, and pyrene. Additionally, we found an increase in heart mass in chicks exposed to phenanthrene, pyrene, chrysene, and fluoranthene at intermediate concentrations. Liver mass increased in embryos exposed to benzo[a]pyrene. Brain mass decreased in embryos exposed to benzo[a]pyrene, phenanthrene, and pyrene. Effects on heart rate and organ mass from embryonic exposure to PAHs could have detrimental effects on hatchling survival and health, especially if these effects contribute to impaired cardiac function post-hatch.

**1.11.P-Tu036 Host-induced gene silencing (HIGS) for agricultural pest control - a promising technology for chemical pesticide reduction**

***Xiaoping Pan****, East Carolina University*

Each year, around two 2 million tonnes of pesticides are applied globally including some persistent chemicals that are not easily biodegradable. Pesticide contaminated soil, water, and air has long been an environmental and human health concern. Efforts are taken to reduce the usage of pesticide through technology development. The host-induced gene silencing (HIGS) technology has been investigated in some commercial crops as a specific and effective way against pest damages\. The HIGS technology use genetic engineered plants that express dsRNA or siRNA, once ingested by pests during feeding on plants, the small RNAs will inhibit specific genes in the pest related to survival, reproduction and development, or parasitism. We will review the current advancement of this technology and its impacts on pesticide reduction, environmental benefits and risks of this technology. The current application of genome-editing technique to generate pest-resistant trait in plants will also be discussed.

**1.11.P-Tu037 Effects of Sodium Benozate on Fat Deposition and Growth In Caenorhabditis elegans**

***Xiaoping Pan****, Leia Lewis and Jerry Vang, East Carolina University*

Clinical observation has proposed the linkage between the occurrence of obesity and slow weight loss with daily exposure to common food additives such as sodium benzoate. However, well-controlled laboratory experiments on this topic have been lacking; the dose-response relationship of sodium benzoate and obesity occurrence and/or slow weight loss has not been established; the potential mechanism of such linkage has not been explored. The insulin-signaling and fatty acid synthesis pathways in the model organism *Caenorhabditis* *elegans* (*C. elegans*) are highly conserved with higher organism including humans and thereby has been widely utilized to study obesity and aging related mechanisms. Scientific literature links the insulin pathway of *C. elegans* to growth, development, longevity, behavior, and metabolism in the organism. *C. elegans* is also a perfect model for exploring the genetics of fat storage. Thereby, *C. elegans* was used as a model organism to study the effects of sodium benzoate exposure on fat storage and on the gene expression of major players in insulin signaling and fatty acid synthesis pathway, as well as, key nuclear hormone receptor genes. This study demonstrated that *C. elegans* growth was greatly affected over time with exposure to sodium benzoate. This study could not provide a clear conclusion about the effects of sodium benzoate on fat deposition. Sodium benzoate exposure also led to the down-regulation of one nuclear hormone receptor gene, and two genes involved in fatty acid synthesis. After 72 hours of exposure, all treatment groups were larger than the control group, with the highest treatment causing significantly more growth. After 72 hours of exposure all treatment groups showed higher fluorescence values than the control group, with the lowest treatment group demonstrating significantly higher fluorescence levels than the control group. Three genes of interest were significantly downregulated after 24 hours exposure: nhr-50, fat-1, and elo-5. These findings indicate that sodium benzoate exposure is significantly impacting the model organism *C. elegans* and could be significantly impacting humans as well.

**1.11.P-Tu038 The Development of a Robust In Silico Bioconcentration Factor (BCF) Model Based on Ionization State Distribution To Address Environmental Concerns of Commercial Chemicals**

***Geetesh Devineni****, Chaitrali Patil, Adelina Voutchkova-Kostal and Jakub Kostal, The George Washington University*

Bioconcentration Factor (BCF) is a key metric in evaluating potential toxicity in the aquatic environment. It is expressed as the concentration of a compound accumulated in an organism vs. the concentration found outside of the organism. Experimentally calculated BCF values are expensive, require long testing periods, and are often highly variable in the outcome based on chosen method and organism. For this reason, Quantitative Structure Activity Relationships (QSARs) are often employed to find relationships between BCF and simple physiochemical properties, such as the octanol-water partition coefficient (Log Po/w), which is a measure of relative lipophilicity with a strong documented correlation to BCF. Here, we describe the development of a BCF model that considers ionization of compounds to address the non-linear response between BCF and physiochemical properties, a notable drawback in existing QSARs. We found that using a predefined cutoff for the most abundant ionized form in conjunction with computed molecular parameters improves the performance of our predictive linear model considerably. Our analysis was carried out using the R statistical software, and relied on mixed quantum and classical mechanics simulations in water to predict physiochemical properties in linear response calculations. We outline the technical details of the development process; which, focused on binning compounds into defined linear ranges, examined trends in BCF values by chemical class, and analyzed outliers for structural clues to explain mechanistic differences. The goal of this effort was to develop a robust approach, which could be used early in the chemical design process and alleviate costs in downstream testing.

**1.11.P-Tu039 Measuring viability and oxidative stress in a humanized Caenorhabditis elegans upon exposure to the environmental pollutant styrene**

*Amy Rand,* ***Amanda Ameyaa-Sakyi*** *and Todd Harris, Carleton University, Canada*

The production and balance of reactive oxygen species is crucial to normal human physiology and is utilized or produced in many processes such as cell signaling and mitochondrial cellular respiration. One issue that arises from over-production of reactive oxygen species is oxidative stress. Chronic oxidative stress has been linked to numerous diseases including asthma, rheumatoid arthritis, cardiovascular issues, and inflammation. Pollutant metabolism and bioactivation can contribute to the formation of reactive oxygen species and oxidative stress.

The human enzyme CYP2E1 facilities the metabolism and occasional bioactivation of small organic pollutants. The aim of this project is to explore whether human CYP2E1 expressed within the nematode *Caenorhabditis elegans* (*C. elegans*) leads to increased oxidative stress upon exposure to styrene as a probe pollutant and CYP2E1 substrate. *C. elegans* viability and response to oxidative stress was visually assessed using microscopy in three traditional methods: survival assays, antioxidant rescue assays, and fluorescent dye (2′,7′-dichlorofluorescein) assays. The survival assays using light microscopy showed a decrease in viability near 99% in 16mM styrene-exposed worms. The antioxidant assays will further strengthen the claim that the observed death is due to reactive oxygen species. In addition, the fluorescence assay may prove to be a more sensitive method, allowing for visualization of reactive oxygen species formation in styrene exposed worms. This assay provides a direct link between styrene exposure and reactive oxygen species overproduction. Results of this study will contribute to the current lethal dose range data regarding styrene and strengthen the mechanistic link between styrene exposure and oxidative stress using a novel humanized animal model.

**1.11.P-Tu040 Evaluation of the in vitro toxicity of indole derivatives: gut bacteria metabolites that may contribute to the etiology of human diseases**

***Maddison Vrazel**, Ramon Lavado and Megan Solan, Baylor University*

Over the past two decades, research interest on the interactions between diet, gut microbiota, and their host organism has grown. New data suggest that tryptophan, an essential amino acid, can be metabolized by microbiota, leading to the synthesis of a biologically active group of indoles. Evidence indicates that indoles derived from gut microbiota metabolism exert significant biological effects. However, most of the research is limited to experimental studies, and most of the data is focused mainly on the actions of indole, and its liver metabolite, indoxyl sulfate. This study was designed to explore the cytotoxic effects of five indole derivatives, indole-3-carboxylic acid (I3CA), indole-3-aldehyde (I3A), indole-3-acetic acid (IAA), indole-3-propionic acid (IPA), and 3-methylindole (skatole, 3-MI), on five relevant human cell lines representing different tissues, the adipose-derived mesenchymal stem cells (MSC), lung fibroblast cells (MRC-5), epithelial cells (T47D), intestinal cells (Caco-2), and the liver cell line HepaRG. The HepaRG cell line exhibits unique qualities; confluent HepaRG cells can be differentiated into hepatocyte- and biliary-like cells maintain many liver-specific functions. Interestingly, while I3CA, IPA, and 3-MI were highly cytotoxic to MSC cells ($EC_{50}$s ranged from 0.2 to 1 µM), they showed low toxicity in hepatocytes ($EC_{50}$s were higher than 50 µM). I3A showed high toxicity in both cell types, but significant differences were observed for IAA: very low toxicity in MSC cells ($EC_{50}$>100 µM) and increased toxicity in HepaRG cells ($EC_{50}$=2.21 ± 0.32 µM). This study evidenced that indole and its derivatives may exert a cytotoxic effect in selected cell types like stem cells, and that effect is indole- and cell-type dependent.

**1.11.P-Tu041 Examining the potential for non-target effects in Florida largemouth bass from aquatic invasive plant management using endothall**

***Joseph H. Bisesi Jr.**[1], Deirdre Honoria Doyle Love[1], Francisco Paneque[1], Katherine Buschang[1], John-Paul Keller[1], Benjamin Sperry[2] and Jason Ferrell[1], (1) University of Florida, (2) U. S. Army Corps of Engineers*

Invasive species are estimated to have cost over $1.2 trillion dollars worldwide over the past four decades. Aquatic invasive plants are among the most common invasive species and present a significant threat to commercial and recreational usage of US waterways. This is especially true in Florida, where aquatic systems are a primary driver of tourism. While aquatic invasive plant management can consist of mechanical or biological control techniques, chemical control via the use of registered aquatic herbicides is most common due to their consistency, selectivity, and efficacy. Despite efforts to ensure treatment of aquatic invasive plants do not cause off target effects in aquatic organisms, there is still tremendous trepidation among the public regarding the potential effects that these herbicides may have on fisheries. Of particular concern are potential off target effects on the Florida Largemouth Bass (FLMB). The FLMB is a prized sportfish across the state as it has both recreational and economic value. While numerous concerns have been expressed regarding the effects of aquatic herbicide use and the potential consequences for FLMB, no comprehensive studies have examined the impacts of this practice on this important Florida sportfish. The objective of this study was to examine the toxicity of the aquatic herbicide endothall on FLMB. Three different life stages (larvae, juveniles, and adults) of FLMB were exposed to endothall using maximum allowable application rates under exposure time scenarios similar to field conditions. Endpoints from experiments will include mortality, growth, histology, plasma hormone concentrations, and molecular markers of effect. Preliminary results from the larval exposure studies indicate no impacts on mortality or growth following at current application rates. Results from the juvenile and adult studies will also be presented. Results from these studies are expected to provide species-specific data that can be used by stakeholders to determine whether current management practices are safe for this recreationally and economically important sportfish.

**1.11.P-Tu042 Examining the potential for disruption of thyroid hormone synthesis by hydroxyl brominated diphenyl ethers through inhibition of negative feedback loops in the hypothalamus**

***Joseph H. Bisesi Jr.***, *Francisco Paneque and Christopher Martyniuk, University of Florida*

Polybrominated diphenyl ethers (BDEs) were used for several years as flame retardants in soft furnishings, and residues remain in the environment, including household dust. While these chemicals have been phased out in the US since the early 2000s, their persistent nature still allows for significant exposure today. Additionally, recent evidence suggests that marine organisms naturally produce BDEs metabolites, which can result in a consistent source of exposure to these chemicals from seafood consumption. Exposure to these chemicals has resulted in measurable levels of BDEs and hydroxylated metabolites of BDEs (OH-BDEs) in body fluids and epidemiological studies have associated these measured compounds with hypothyroidism. Several studies have also indicated that OH-BDEs are associated with thyroid system toxicity due to their structural similarity to thyroid hormones and affinity for thyroid receptors. It is important to point out that to date, these studies have focused almost exclusively on the interactions of OH-BDEs with thyroid receptors that are involved in processes that are downstream of thyroid hormone synthesis, however, few studies have examined the role of these chemicals on the production of thyroid hormones. We hypothesize that OH-BDE interacts with the thyroid hormone receptor beta 2 (TRβ2), a thyroid receptor isoform found exclusively in the hypothalamus and pituitary where it is involved in feedback inhibition of thyroid hormone synthesis. In this study we will test this hypothesis using receptor transactivation assays with TRβ1 and TRβ2. Our preliminary data indicates that OH-BDEs are able to antagonize TRβ2, which controls circulating thyroid hormone levels by inhibiting Thyrotropin-releasing hormone (TRH). In additional experiments that will be presented, we will utilize a hypothalamus cell line to test whether interactions with TRβ2 impacts the production of TRH which controls the release of thyroid hormones from the thyroid gland. Results from these studies will provide evidence for a novel mechanism by which PBDEs can directly influence circulating thyroid hormones. These data are essential for providing a mechanistic link between exposure data for PBDEs and epidemiological evidence associated hypothyroidism.

**1.11.P-Tu043 Assessing the Effects of the Pharmaceutical Contaminant Atorvastatin on the Growth and Metabolome of the Red Swamp Crayfish, Procambarus clarkii**

***Robert Charles Dixon***[1]*, Frank Bailey*[1]*, Steven Symes*[2] *and Sean Richards*[2]*, (1) Middle Tennessee State University, (2) University of Tennessee at Chattanooga*

Human pharmaceuticals are emerging contaminants of great concern throughout the world. One such pharmaceutical, atorvastatin (Lipitor), is one of the most prescribed drugs in the United States and poses a potential threat to aquatic invertebrates, such as crayfish, due to its presence in wastewater treatment plant effluent. Crayfish (Astacoidea and Parasticoidea) are considered keystone species in many environments, and several species are threatened throughout their range. Statins act through the inhibition of hydroxymethylglutaryl-CoA-reductase (HMGR) in the mevalonate pathway, which has been shown to inhibit the juvenile growth hormone, methyl farnesoate, in some arthropods. In this present study, red swamp crayfish (*Procambarus clarkii*), raised in a controlled lab setting over the period of eight weeks, will be dosed with 4 different concentrations (0.15, 1.5, 15, and 150 µg/L) of atorvastatin calcium in conditioned tap water, along with solvent and sans solvent control groups. 50 crayfish will be sacrificed after the study period, having tail and hemolymph samples taken for analysis. To determine the effects that atorvastatin may have on this group of organisms, the growth, metabolome, methyl farnesoate levels, and chitobiase (molting) activity will be assessed in the whole crayfish, tail tissue, hemolymph, and water respectively. Chitobiase activity was measured after the first 4 weeks and was found to not differ significantly across treatments and controls (Kruskal-Wallis chi-squared = 8.3009, df = 5, p = 0.1404). Values ranged from 324.096 nM/ hr to 686,352.786 nM/ hr, with the 1.5 µg/L dosing group having the highest average activity at 1,935.350 nM/ hr. Molting frequency, weight, and length also do not appear to vary across the dosing groups after 4 weeks, as molting did not occur in this time period. Overall, the effects measured here will help to inform future studies about threats crayfish face in the aquatic environment.

## 1.11.V Poster Only: Environmental Toxicology and Stress Response

### 1.11.V-02 Benzo[a]pyrene-7,8-diol-9,10-epoxide Impacts Gene Silencing Through Inactivation of Epigenetic Enzymes

***Kuelye Lee****, Daniel Sanmiguel, Cesar Marquez and Frauke Seemann, Texas A&M University, Corpus Christi*

The environmental pollutant, benzo[a]pyrene (BaP), found in tobacco and petroleum products, has been shown to induce transgenerational toxicity in laboratory fish. Inheritance of a modified epigenetic pattern through the germ line is suspected to be the underlying mechanism of BaP-induced transgenerational phenotype. The BaP metabolite Benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE) is suspected to induce histone demethylation, and thus, likely to activate gene expression. To elucidate the interaction of BPDE with epigenetic mechanisms, in silico docking and molecular dynamics analysis were conducted for a histone methyltransferase and a DNA methyltransferase. The active domains of the polycomb repressive complex 1 and 2 (PRC1; PRC2) and DNA methyltransferase 3 A and B (DNMT3A; DNMT3B) are both involved in gene silencing and were examined in this study. PRC1/2 catalyze the methylation of lysine 27 on histone 3 with a major role in cell fate decisions, while DNMT3A/B regulate de-novo DNA methylation in germ cells and maternal imprinting in the embryo. Our results indicate that BPDE tends to dock stereospecific in the enzymatically active domain (EED WD40) of PRC1/2, while docking was not stereospecific for the two other catalytic core regions (SET; SANT2L), which are required for binding to the histone 3 tail and methyltransferase activity. For DNMT3A/B, BPDE docking occurs in the catalytic loop near the target recognition domain putatively resulting in a modified CpG nucleotide recognition. Future research will focus on improving the accuracy of the results by conducting replica exchange molecular dynamics using the GROMACS 5.0 package and confirmed by using the ANTECHAMBER package from Amber Tools. The present results suggest an active interference of BPDE with both epigenetic enzymes possibly entailing transmission of a modified epigenetic profile through the germ line upon parental BaP exposure. This study sheds light on the potential mechanisms of ancestral BaP exposure-induced impacts on the phenotype inheritance in the offspring and further highlights the necessity of a refined risk assessment for BaP.

## 1.12.P Late Breaking Science: Environmental Toxicology and Stress Response

### 1.12.P-Mo179 Tissue Metal Concentrations and Antioxidant Enzyme Activity in North Atlantic White Sharks

***Gretchen K. Bielmyer-Fraser**[1], Bryan Franks[1], Rachel Somerville[1] and Chris Fischer[2], (1) Jacksonville University, (2) OCEARCH*

Metals occur naturally in the environment; however, anthropogenic practices have resulted in increased metal concentrations in coastal ecosystems. Sharks are important species, ecologically, recreationally and commercially. Reference levels of metal contaminants in the tissues of sharks, particularly, great whites, is lacking. In this study, concentrations of copper, cadmium, nickel, lead, selenium, silver, and zinc were measured in the muscle tissue of great white and tiger sharks. Metal exposure in various species has been correlated with oxidative stress. Therefore, activities of antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase) were also examined in the shark muscle tissue with the objective of identifying a nonlethal bioindicator of metal pollution. Metal-specific differences in tissue metal concentrations were detected based on collection site for copper, zinc, and nickel, and age class and sex for nickel, with females having higher nickel levels. This is the first study to report data on metal concentrations and antioxidant stress enzymes in the muscle tissue of North Atlantic white sharks and provides insight into oxidative stress defenses in these top-level carnivores.

**1.12.P-Mo180 Assessing the Quality of Emergent Adverse Outcome Pathways Using Semantic Analysis**

***Nathan Pollesch**, Jennifer Olker and Rong-Lin Wang, U.S. Environmental Protection Agency*

Contribution, collaboration, and curation of the Adverse Outcome Pathway knowledgebase has enabled computational knowledge discovery research that utilizes the information within the AOP knowledgebase. One active area of research is the discovery and identification of Emergent Adverse Outcome Pathways (emAOPs). The creation of emAOPs is a result of key event sharing by AOP authors and network analytic techniques provide methods by which emAOPs can be identified. Previous research has shown that thousands of emAOPs exist in the AOP-Wiki. Given the large number of emAOPs, computational methods must be developed to assess their biological integrity and validity. Semantic coherence analysis has been shown recently to be a useful technique to compare and assess the quality of AOPs. In this presentation, methods and results from the semantic coherence analyses of emAOPs using a variety of newly developed semantic similarity metrics are shared. Our results indicate that many emAOPs are of high semantic quality, and we discuss the benefits and drawbacks of different metrics for prioritizing high quality emAOPs for expert review. The combination of emergent AOP knowledge discovery methods using network analysis and biological quality assessment using semantic analysis is demonstrated as a promising combination of methods to a workflow that can take advantage of, and contribute to, the AOP knowledgebase.

**1.12.P-Mo181 Development and Applications of a Zebrafish (Danio rerio) CYP1A-Targeted Monoclonal Antibody (CRC4) with Reactivity across Vertebrate Taxa: Evidence for a Conserved CYP1A Epitope**

*Amy L Anderson[1], Benjamin D Dubansky[2], Lindsay B Wilson[3], Robyn Leigh Tanguay[3], Alyssa M. Whisel[1] and **Charles D. Rice**[1], (1) Clemson University, (2) Louisiana State University, (3) Oregon State University,*

CYP1A is a heme-thiolate enzyme associated with the cytochrome P4501A1 monooxygenase system and is inducible by a wide variety of xenobiotics and endogenous ligands that bind and activate the aryl hydrocarbon receptor (AHR). The AHR-CYP1A axis is important for detoxification of certain xenobiotics and for homeostatic balance of endogenous sex hormones, amine hormones, vitamins, fatty acids, and phospholipids. Herein, we generated and described applications of a zebrafish CYP1A-targeted monoclonal antibody (mAb CRC4) that fortuitously recognizes induced CYP1A across vertebrate taxa, including fish, chicken, mouse, rat, and human. We then demonstrated that mAb CRC4 targets a highly conserved epitope signature of vertebrate CYP1A. The unique complimentary determining region (CDR) sequences of heavy and light chains were determined, and these Ig sequences will allow for the expression of recombinant mAb CRC4, thus superseding the need for long-term hybridoma maintenance. This antibody works well for immunohistochemistry (IHC), as well as whole-mounted IHC in zebrafish embryos. Monoclonal antibody CRC4 may be particularly useful for studying the AHR-CYP1A axis in multiple vertebrate species and within the context of Oceans and Human Health research. By using archived samples, when possible, we actively promoted efforts to reduce, replace, and refine studies involving live animals.

**1.12.P-Mo182 Metabolic Profiling and Toxicity Analysis of Mahi-mahi Embryos Exposed to Brevetoxin Using High-resolution Magic-angle Spinning Nuclear Magnetic Resonance (HRMAS NMR)**

***Ariel Lawson**[1], Mark Annunziato[1], Muhamed Eeza[2], Daniel Benetti[3], Martin Grosell[3], John Stieglitz[3], Alia Alia[2] and John Berry[1], (1) Florida International University, (2) Leipzig University, Germany, (3) University of Miami*

There is widespread agreement that brevetoxins (PbTx), a class of neurotoxins, are responsible for the devastation of marine ecosystems and human health caused by hazardous algal blooms. Most notably, the periodic Florida red tides that occur in coastal waters result in massive fish kills, poisoning from eating neurotoxic shellfish, and respiratory distress; all indicate that Florida red tides induce significant neurotoxicity. To better understand the environmental implications of PbTx, it is necessary to understand the mechanisms and pathways of toxicity. High-resolution magic angle spinning nuclear magnetic resonance (HRMAS NMR) was used for metabolic profiling of mahi-mahi (*Coryphaena hippurus*) embryos exposed to PbTx-2 based on established zebrafish (*Danio rerio*) models to get fundamental metabolic knowledge of toxicity and identify possible biomarkers. Mahi-mahi represents a biologically relevant marine fish species because of its proximity to the red tides. Toxicity testing was performed to determine its $LD_{50}$, and based on those concentrations, and embryonic exposures were performed at parts per billion concentrations. The metabolomics studies involved exposing intact mahi-mahi embryos to sub-lethal amounts of PbTx-2, and subsequent HRMAS NMR analysis yielded well-resolved spectra, allowing for the identification of 36 metabolites. Those involved in glycolysis and the citric acid cycle were significantly changed compared to controls. The pathways involving glucose and energy metabolism affected by PbTx-2 exposure are those linked with neuronal excitotoxicity. By measuring many metabolites, metabolomics provides an in-depth and retrospective description of previous biological activity within an organism, thus allowing for an understanding of the larger picture of PbTx toxicity. This provides more insight into the metabolic targets and mechanisms of PbTx, resulting in discovering potential biomarkers applicable to toxicometabolomic assessment and approaches.

**1.12.P-Mo183 Developing an Assay to Assess Neurobehavioral Impacts of Environmental Contaminants in Fathead minnows (Pimephales promelas)**

***Vaibhavi Srinivasan**, Alexis Khursigara and Aaron Roberts, University of North Texas*

Adverse effects on the neurobehaviors of fishes due to environmental toxicants can have a serious impact in their cognitive abilities to learn and memorize. Cognitive processes like learning and memory are important for fishes as they have an important role in predator avoidance, identifying mates, accessing food and for spatial orientation. Associating a particular cue to a negative stimulus and remembering it, aids the fish in avoiding threats in their environments. Neurobehavioral assays in fishes have been developed and widely used to evaluate the effects of various environmental toxicants that can impair motor and cognitive processes. This learning assay used a T maze to study learning behaviors in Fathead minnows (*Pimephales promelas*) and to determine if this was a viable learning assay for minnows. Fish were subjected to 5 days of training in the T maze where one end was a positive stimulus zone (fish received food reward) and the other end was a negative stimulus zone (water agitation). Both of the zones were marked with a visual cue. Time taken to reach the positive zone, number of visits to both zones and time spent in both zones were recorded for pre and post training. Post training, fish avoided the negative stimulus zone altogether and preferred to be near or in the positive stimulus zone. Reductions in the number of visits and time spent in the negative zone demonstrate that the fish has learned to associate visual cues with the positive and negative zones. This preliminary data further shows that this is a viable assay to test learning in fathead minnows and thus suggests that this assay can be applied in ecotoxicological fields to determine neurobehavioral toxicities of different environmental toxicants.

**1.12.P-Mo184 Mycoremediation of Petroleum Crude Oil Enhancement with Nitrogen and Phosphorus Amendments**

***Summer S Crescent**[1], Emily C. Pisarski[2] and Ed Wirth[2], (1) Florida A&M University, (2) National Oceanic and Atmospheric Administration (NOAA)*

*Pleurotus ostreatus* (oyster mushroom) is a white rot fungus known to biodegrade recalcitrant molecules such as polycyclic aromatic hydrocarbons (PAH) in petroleum crude oil, which can be both toxic and persistent in the coastal environment. Restoration efforts for oil spills in estuarine environments impose complex problems due to the sensitivity of the environmental resources at risk, which leads to clean up methods that can cause detriment to ecosystems. *P. ostreatus* has been proposed as a bioremediation tool for oil spill response. We are evaluating the ability of *P. ostreatus* to mineralize the saturate, aromatic, resin, and asphaltene (SARA) fractions constituting crude oil and the influence of various estuarine environmental parameters on degradation efficiency. This work examined in vitro mycelium growth response with influence of nitrogen and phosphorus amendments based on this species' C:N:P ratio. We used imaging analysis to measure the growth rate in response to crude oil and nutrients, then applied SARA analysis to the oil extractions. Oil spills can be devastating to coastal ecosystems and can negatively impact the blue economy and human health. With added knowledge about the efficiency of oyster mushrooms to biodegrade petroleum crude oil, they may be used in the future as an effective remediation tool.

**1.12.P-Mo185 Combined Effects of Microplastics (PET) and Food Availability on the Life History Traits of Daphnia magna**

***Safa Chaabani***, *Norwegian University of Science and Technology (NTNU)*

Microplastics are considered among the emerging contaminants of concern, due to their increasing consumption and their persistence in marine and freshwater ecosystems. After fragmentation, they can easily disperse in the environment and are often mistaken as food by small organisms, especially non-selective filter feeders. Particularly, Polyethylene terephthalate (PET), which is mainly used as packaging material, is one of the most produced polymers and accounts for a high proportion of total plastic consumption. To date, information about the mechanism of effects of PET on aquatic organisms is limited. Dynamic Energy Budget (DEB) models are flexible mechanistic tools that can be applied to understand toxic effects and extrapolate individual responses to higher biological levels and under untested environmental conditions. In this work, we used the DEBtox (an application of the DEB theory to ecotoxicology) approach to investigate the possible physiological mode of action of PET fragments (<63 µm) and the combined effects of PET and food availability on the model organism *Daphnia magna*. Our preliminary results revealed an increasing negative effect PET on the reproduction, growth and survival of the animals with increasing concentration (125, 250 and 500 mg/L). The impacts were stronger under limited food conditions. The study will allow to extrapolate the results to other species and other exposure situations, which depends critically on the selected mode of action. The results will be further used to investigate the population response to PET combined with other environmental stressors such as temperature and trophic control.

**1.12.P-Mo186 The Combined Effects of UV Radiation and Crude Oil Thin Sheens on Invertebrates and Fish**

*Aaron Roberts, Rachel R. Leads and* ***Abby Chapman****, University of North Texas*

Chironomidae, a family of aquatic insects within the order Diptera, are the most broadly distributed holometabolous insects, and are among the most abundant and widespread aquatic insects on Earth. Collecting and identifying benthic macroinvertebrates are standard practice in freshwater bioassessments, due to the abundance of taxon-specific toxicity data regarding sensitivities to a variety of pollutants. Chironomidae often dominate assemblages in areas where petroleum-derived polycyclic aromatic hydrocarbons (PAHs) are present. However, lab studies often conduct single PAH sediment tests when determining PAH toxicity, and the increase in PAH toxicity when exposed to UV radiation (phototoxicity) is often excluded. Oil can enter freshwater environments through stormwater runoff to create thin sheens of oil on surface water, and PAHs partition from the sheen onto sediments. To our knowledge, this study is the first to assess sensitivity of Chironomus dilutus to the phototoxicity of thin sheens of crude oil. Chironomus dilutus were cultured in the laboratory and exposed as ten day old (third instar) larvae. Twenty individuals each were placed in 250 mL crystallizing dishes along with sediment and culture water. Organisms were exposed to one of four treatments: UV radiation, Oil Sheen, UV radiation and oil sheen, and a control. This test was performed with silica sand and then repeated with a fine sand with varying water depths to bring the sheen closer or farther from the sediment. There were no significant differences in mortality in Chironomus dilutus on sediments. This is in contrast to studies done with thin sheens and pelagic organisms such as fish and daphnia. These data suggest that sediment dwelling organisms may be at low risk for photo-toxicity following thin sheen exposure.

**1.12.P-Mo187 Impacts of Lead (Pb) on Development and Behavioral Response to Acute Stressors in Early-Developing Zebrafish**

***Ahmed Abdelmoneim*** *and Emily Savoie, Louisiana State University*

Lead (Pb) is a ubiquitous environmental contaminant and neurotoxin linked to various adverse health effects. However, the impacts of early exposure to Pb on the behavioral stress response, a system that plays a critical role in adaptation and survival, are largely unknown. In this study, we investigated the developmental changes and alterations in the behavioral response to acute stressors in larval zebrafish following Lead (II) Acetate exposures that extended between 6 and 120 hours post-fertilization. Zebrafish embryos were exposed to concentrations corresponding to the Maximum Contaminant Level (MCL) for Pb in drinking water as set by the US EPA (15 mg/L) and folds higher (5, 10, 25, and 50-fold). At the end of the exposure period, the effects on survival, development, activity, and behavioral response to acute stressors were assessed. Exposure to concentrations corresponding to 25 and 50-fold the MCL significantly reduced the survival of zebrafish larvae. The overall larval development, assessed by measuring total larval length, was not significantly altered. Exposure to Pb concentrations at MCL and folds higher resulted in a significantly higher prevalence of deformities in the body axis, snout, jaw, brain, pericardium, heart, and swim bladder. The behavioral response to visual or acoustic stimuli (acute stressors) was significantly attenuated following exposures to Pb concentrations at MCL and folds higher. Exposure to peripheral irritants, however, significantly activated the larval behavioral response. Our findings shed light on the impacts of early-life exposure to environmentally relevant concentrations of Pb on the behavioral response to acute stressors and highlight the need for further investigations to understand the underlying changes in the stress circuitry.

**1.12.P-Mo188 The Synthetic Phenolic Antioxidant Tert-Butylhydroquinone (TBHQ) is a potent Immune Disrupting Compound in Macrophages: A possible role of ACOD1/Irg1 in Sensitization**

***Alyssa M. Whisel*** *and Charles D. Rice, Clemson University*

The field of immunotoxicology has demonstrated that several environmental contaminants target the immune system of both environmental species and humans. Iconic and well-studied immunotoxic compounds include benzo-*a*-pyrene, PCB-126, TCDD, select heavy metals, and more recently several classes of emerging contaminants of concern are being investigated. To this end, we herein employed the murine macrophage cell line RAW264.7 in screening assays that possibly predict immunotoxicity in other cell types and at higher immune functions. This cell line is very well characterized and a common in vitro model system for understanding macrophage-related inflammation profiles in immunotoxicology and immunopharmacology. Specifically, we used inducible nitric oxide synthase (iNOS) to screen many common environmental contaminants for the ability to modulate iNOS activity as a biomarker of pro- or anti-inflammatory properties. One class of compounds, the synthetic phenolic antioxidants (SPA), proved to be strong modulators of iNOS activity, and of these tert-butylhydroquinone (TBHQ) is the most potent. Because TBHQ is a common antioxidant food additive, it is usually not associated with environmental matrices at the same level of concern as other SPAs. However, the impact of TBHQ on fish and wildlife is virtually unknown. Other studies show that TBHQ activates transcription factors AHR and NRF2, may be carcinogenic at high levels, and is immunotoxic in rodent studies. In our study, TBHQ was immunotoxic at levels as low as 2 micromolar in both LPS- and poly I:C-stimulated macrophages. This level of activity is comparable to the effects of indirubin E804, a potent anti-inflammatory compound, and a positive control for this study. To aid in our studies, a monoclonal antibody (AW12) was generated against ACOD1/IRG1 (immune response gene) and used to demonstrate that macrophages lacking IRG1 are more sensitive to TBHQ, suggesting a protective role of IRG1. IRG1 is a newly discovered gene product involved in macrophage immunometabolism and has a key role in regulating macrophage activation. Based on these data, further studies are underway to determine if SPAs, such as TBHQ, polarize macrophages from a classically activated state towards an alternatively activated state. If so, then this may be a common, but understudied mechanism of action.

**1.12.P-Mo189 PFAS in The Chesapeake Bay and Delmarva Region**

***Michella Salvitti****, Eguono Wayne Omagamre and Joseph Pitula, University of Maryland Eastern Shore*

The group of per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals that enter ecosystems most frequently through landfills, wastewater treatment plants, PFAS based pesticides, and industrial sites. In recent months, the Environmental Protection Agency (EPA) updated the PFAS drinking water health advisory and lowered the acceptable Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) levels exponentially than what they were previously. We investigated the levels of PFAS present in the Delmarva peninsula and Chesapeake Bay by collecting soil, ground water, and Bay water samples. In the Delmarva region, soil and groundwater samples were collected at 8 farms and 3 non-farm sites. Results detected a presence of PFAS in both soil and groundwater. Of the 8 farm sites, 36.5% detected at least 1 PFAS in the groundwater samples and 75% of farm sites detected at least 1 PFAS in the soil. All of the non-farm sites identified 7 or 8 PFAS. The 9 sites that detected PFAS in their soils all had PFOS. When evaluating the Chesapeake Bay waters, sites were selected by their proximity to land use areas that are known to have PFAS activity. Surface water samples were collected where the Potomac River meet the bay, a site with military and industrial activity, a site with high human and industrial activity, and the fourth site was in a river with landfill and agriculture activity. Results for relative PFAS concentration in Chesapeake Bay surface water and marine soil samples will be presented.

**1.12.P-Mo190 Realistic Exposure of Pollinators to Airborne Pesticides: Are Current Contact Toxicity Methods Indicative of Environmental Exposure Scenarios?**

***Frank B Green*** *and Philip Smith, Texas Tech University*

Current pollinator contact toxicity test methods call for application of neat pesticides dissolved in solvents directly to the test subject. Unfortunately, these methods may not be reflective of realistic environmental conditions faced by pollinators in the wild. Further, recent data suggest differences in toxic effects of pesticide classes between pollinator species, but US EPA and OECD protocols are typically only conducted with standardized model species (e.g., honeybees or bumble bees). Thus, standard pollinator toxicity tests with only honeybees and/or bumblebees may be inadequate for some exposure scenarios. As an example, numerous pesticides and veterinary pharmaceuticals bound to airborne particulate matter (PM) have been detected in environs surrounding agricultural operations (e.g., beef cattle feedyards). Herein we used a recently developed, laboratory-based pollinator toxicity test method to simulate real world pollinator exposure to pesticide-laden PM. Honeybees, mason bees, and painted lady butterfly larvae were subjected to exposures to PM spiked with individual agrochemicals – permethrin, bifenthrin, ivermectin, abamectin, clothianidin, imidacloprid, thiamethoxam, and fenbendazole. Exposure concentrations were based on recently reported mean and maximum concentrations of each chemical in fugitive beef cattle feedyard PM, and a control group was exposed to agrochemical-free PM. Honeybees experienced significantly higher mortality when exposed to PM spiked with permethrin or bifenthrin, as well as all three neonicotinoids. There was no significant increase in morality of honeybees exposed to PM spiked with ivermectin, abamectin, or fenbendazole. Mason bees and painted lady larvae experienced significantly higher mortality when exposed to PM spiked with each of the eight chemicals. These data indicate pollinator morality from insecticides and parasiticides bound to PM, but to a different extent than those of standard toxicity test methods, depending on the chemical and pollinator species. For example, standard contact methods using honeybees have estimated an $LD_{50}$ of approx. 63 ng/bee, while this study found approx. 36% mortality at 75 ng/bee, indicating variation in toxic responses due to changes in exposure method. Additionally, standardized methods may not accurately predict toxicity to non-*Apis* pollinator species; our data indicate that mason bees are less sensitive to pyrethroids bound to PM than honeybees.

**1.12.P-Mo191 Cohesin Malfunction, a New Mechanism for Hexavalent Chromium-Induced Carcinogenesis**

***Idoia Meaza****, Jennifer Toyoda, Joseph Calvin Kouokam, Caitlin R. Cahill and John P. Wise, University of Louisville*

Hexavalent chromium [Cr(VI)] is an environmental and occupational lung carcinogen. Although it is not completely known how it induces cancer, chromosome instability is central to its carcinogenic mechanism. Cohesin, a ring protein complex, is involved in genomic stability by keeping sister chromatid cohesion at chromosomal arms and centromeres, centriole cohesion and regulates chromatin topology at the DNA loop level. Thus, it is well-known that cohesin malfunction can lead to chromosome instability. Here, we hypothesize Cr(VI) exposure leads to the malfunction of cohesin by targeting 4 key regulatory proteins in the unloading of cohesin; separase, PDS5A & B, and WAPL**.** PDS5A & B and WAPL remove cohesin from chromosome arms during prophase, while separase removes it from the centromere and centrioles during anaphase. To test this, human lung cells were exposed to various zinc chromate concentrations for 24, 72 and 120 h. Chromosome aberration assay was performed to measure cohesin malfunction at metaphase. We identified 120 h exposure to Cr(VI) increased premature anaphase, premature centromere division and centromere spreading, all effects of cohesin malfunction. To measure cohesin malfunction at the centriole and centrosome level, immunofluorescence methods were used, and we showed aberrant centrosome amplification and centriole disengagement increased at >72 h exposures. The effects of Cr(VI) in the key 4 regulators of cohesin were measured by Western blot and RNA-seq analyses. Our results show, active separase increased after 120 h Cr(VI) exposure, measured by cleaved levels, suggesting overactive separase might prematurely cleave cohesin at the centromere and centrioles, leading to the aberrant phenotypes mentioned above. PDS5A & B protein levels increased after 24 h of Cr(VI) exposure, but this effect was lost after 120 h, suggesting cohesin unloading from the chromosome arms might be impaired. Although the mRNA levels followed those trends, the changes were not statistically significant. WAPL protein and mRNA levels did not change; however, Sororin, a protein that antagonizes WAPL and increases cohesin complex stability, was greatly inhibited at the mRNA level, suggesting lack of Sororin might be destabilizing the cohesin complex. All in all, Cr(VI) targeted key unloaders of cohesin, which can lead to numerical chromosome instability, a key step in Cr(VI)-carcinogenesis. Funding: NIEHS [R01ES016893, R35ES032876] (JPW) and NCI R25CA134283 (CRC).

**1.12.P-Mo206 Physiological oxygen tension is critical for predictive in vitro toxicology testing and risk assessment**

***Shana Rogan*** *and Michael Laiosa, University of Wisconsin, Milwaukee*

Most *in vitro* toxicological studies are conducted in environmental chambers purported to maintain conditions identical to the human body but fail to account for tissue specific $O_2$ gradients. Thus, cells are exposed to severely hyperoxic conditions relative to *in vivo*. These hyperoxic conditions may impair cellular functions due to increased production of reactive oxygen species (ROS); these effects are non-reversible and are often initiated from only brief exposure to ambient air (21% $O_2$). Exposure to ambient air may be masking nuanced effects that could be detrimental to physiological systems such as the immune system. Hence, our objective was to develop an *ex vivo* culturing method using primary cells that is more physiologically relevant to assess the immunotoxicity potential of environmental toxins and toxicants. Specifically, we focused on hematopoiesis as a functional endpoint, an essential process for multi-lineage blood cells generation and immune system function. For these studies, a culturing system that promotes a 200-800-fold expansion of murine hematopoietic stem cell (HSC) and hematopoietic progenitor cells in one month was adapted for physioxic growth conditions (5% $O_2$). As a first step to mitigate elevated ROS that results from high oxygen tension during cell harvesting and preparation, we supplemented the cell preparation media with cyclosporin A, a compound that prevents the rapid production of ROS. Using cyclosporin A and 5% $O_2$ we found that approximately 40% more HSCs were produced on day 12 of culture compared to cultures grown in a traditional incubator (21% $O_2$). As a proof of principle testing our hypothesis that $O_2$ tension impacts sensitivity to potential toxicological impacts we exposed HSC cultures grown in either 21% or 5% $O_2$ to the environmental pollutant and well characterized toxicant *2,3,7,8 – Tetrachlorodibenzo-p-dioxin* (TCDD) across a range of exposures from 0.001 to 0.1 nM. We found that cultures grown at 5% $O_2$ were sensitive to the lowest dose (0.001 nM), indicated by an approximately 20% reduction in total cell number (p=0.024) and 20% reduction of non-committed hematopoietic precursor cells. In contrast, cultures grown at 21% $O_2$ were not sensitive to 0.001 nM TCDD. Our data highlights the importance of conducting *in vitro* toxicological studies using physioxic growth conditions to better model potential mechanistic impacts of *in vivo* toxic insults on human health.

## 1.12.V Late Breaking Science: Environmental Toxicology and Stress Response

### 1.12.V-01 Utilization of piperonyl butoxide and 1-aminobenzotriazole for metabolic studies of toxic chemicals in aquatic arthropods

***Shinpei Ohnuki***[1]*, Yoko Osawa*[1]*, Takeru Matsumoto*[1]*, Shinichi Tokishita*[2] *and Shoko Fujiwara*[2]*, (1) Nippon Soda Co., Ltd., Japan, (2) Tokyo University of Pharmacy and Life Sciences, Japan*

Daphnids and chironomids are used to assess ecological effects of chemicals released into the water body. However, the toxicity mechanisms of xenobiotic chemicals in the organisms are generally difficult to be identified. Here, we developed a test system capable of estimating the contribution of cytochrome P450 (CYP) to the metabolism of test substances in *Daphnia magna* and *Chironomus yoshimatsui*, based on the difference in toxicity in the absence and presence of the CYP inhibitors piperonyl butoxide (PBO) and 1-aminobenzotriazole (ABT), applying previous reports on the inhibition of insecticide metabolism by CYPs using PBO in several aquatic species. The optimum concentrations of PBO and ABT effective for reduction of the toxicity of diazinon, which is toxic after oxidative metabolism *in vivo*, were determined. The concentrations of PBO and ABT were 0.5 mg/L and 0.6 mg/L for *D. magna*, and 2.0 mg/L and 40.0 mg/L for *C. yoshimatsui*, respectively. As the results of acute immobilization tests of 15 insecticides with and without the optimum concentrations of PBO or ABT, chlorpyrifos and chlorfenapyr toxicities were reduced in both organisms, while those of thiocyclam, nereistoxin, and silafluofen were enhanced in *C. yoshimatsu*, in the presence of either inhibitor. LC-MS analysis of *D. magna* and *C. yoshimatsu* exposed to chlorfenapyr confirmed that the level of the active metabolite produced by CYP was clearly decreased by PBO or ABT in both organisms. The test system in which the test substance was co-exposed with PBO or ABT, will be valuable in estimating the contribution of CYPs to metabolism, and likely will be applicable to elucidate the toxicity mechanism in daphnids and chironomids.

### 1.12.V-02 Adverse Outcome Prediction of Oncogenic Mycotoxin Stress via miRNA Target-based Androgen Receptor-responsive Network

*Arulkumar Nagappan and* ***Yuseok Moon****, Pusan National University, Korea, Republic of (South)*

Stress-responsive microRNAs (miRNAs) contribute to the regulation of cellular homeostasis or pathological processes, including carcinogenesis, by reprogramming target gene expression following human exposure to environmental or dietary xenobiotics. Herein, we predicted the targets of carcinogenic mycotoxin-responsive miRNAs and analyzed their association with disease and functionality using network modeling integration. miRNA target-derived prediction indicated potent associations of oncogenic mycotoxin exposure with metabolism- or hormone-related diseases, including sex hormone-linked cancers. Mechanistically, the signaling network evaluation suggested androgen receptor (AR)-linked signaling as a common pivotal cluster associated with metabolism- or hormone-related tumorigenesis in response to aflatoxin $B_1$ and ochratoxin A co-exposure. Particularly, high levels of AR and AR-linked genes for the retinol and xenobiotic metabolic enzymes were positively associated with attenuated disease biomarkers and good prognosis in patients with liver or kidney cancers according to clinical transcriptome datasets. Moreover, AR-linked signaling was protective against OTA-induced genetic insults in human hepatocytes whereas it was positively involved in $AFB_1$-induced genotoxic actions. Collectively, miRNA target network-based predictions provide novel clinical insights into the progression or intervention against malignant adverse outcomes of human exposure to environmental oncogenic insults (This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2021R1I1A1A01056963)).

### 1.12.V-03 Using Machine Learning algorithm to predict CO2 during the Cement Manufacturing Process: A case study at Union Bridge Plant, Lehigh Hanson, Maryland.

***Kwaku Boakye**, Kevin Patrick Fenton, Timi Oguntola and Steve Simske, Colorado State University*
This study is ongoing and aims to improve the understanding of the generation of CO2 during cement manufacturing by modeling the calcination process in cement using machine learning algorithms. Characterizing the clinker quality, energy requirements, and CO2 emissions in a cement manufacturing facility heavily relies on calcination. It has always been difficult to accurately predict the $CO_2$ generated during the calcination process due to its complexity. This study will attempt to access $CO_2$ related to raw material generation and the final grinding of the clinker to form cement.

In this study, six machine learning algorithms will be tested to analyze two output variables, which are, 1). the apparent degree of calcination, and 2). $CO_2$ molar fraction (dry basis). Over six thousand input variables data obtained from historic manufacturing health data will be used for sensitivity analysis and sensitivity analysis output will be used to train the algorithms. Various regression models will be analyzed for analysis on which will provide the lowest Root Mean Squared Error, and then based upon these results, run the models to determine which cement manufacturing independent variables had the greatest influence on the dependent variables. The importance of the independent variables will also be evaluated to determine which have the greatest influence upon the $CO_2$ emissions. Predicated $CO_2$ data will be compared with empirical results obtained using the IPCC, 2006 equations.

## Track 2: Aquatic Toxicology, Ecology and Stress Response

### 2.01.P Advances in the Ecotoxicology of Reef-building Corals

#### 2.01.P-Mo031 Effects of Sedimentation on Three Hawaiian Coral Species under Laboratory Conditions

***Justin Wilkens**[1], Alexandria Barkman[2], Alexi Meltel[2], Burton Suedel[1] and Robert H. Richmond[2], (1) U.S. Army Corps of Engineers, (2) University of Hawaiʻi at Mānoa*
Sedimentation can occur near a dredge operation in pulses over days, and potentially impact coral reefs that occur in close proximity. To improve the ability to predict the effects of dredging on coral reefs, the effects of sedimentation in two 18-d experiments were determined for three common coral species representing different morphologies exposed to sediment concentrations (range 0 to 60 $mg^{-1}$ $cm^{-2}$) dosed every 4 days. The experiment was performed once with fine grain sediment and repeated with a coarse grain sediment. A 30-d sediment free observation period followed each experiment. Photosynthetic yield, lipid ratios, tissue color, tissue loss, growth, and sediment coverage varied among the treatment groups. *Pocillopora meandrina* and *Porites lobata* experienced the most sediment cover and tissue loss when exposed to sediment concentrations >30 $mg^{-1}$ $cm^{-2}$ for either sediment. *Montipora capitata* experienced relatively no sediment coverage or tissue loss when exposed to either sediment, but a reduction in photosynthetic yield at 60 $mg^{-1}$ $cm^{-2}$ fine grain sediment was observed. During the 30-d post-exposure observation period, *P. meandrina* tissue loss continued, *P. lobata* resumed tissue growth, while *M. capitata* showed no lingering effects. This study improves the ability to estimate the impacts of dredging on coral reefs.

#### 2.01.P-Mo032 Towards the development of standard toxicity test protocols for corals

***Guido Gonsior** and Maren Dill, GG BioTech Design, Germany*
Chemical pollutants affect corals at very low concentration levels. In focus are skincare chemicals. Beside this increasing run-off of pesticides from agriculture and industrial wastewater are further origins for contaminants in the costal water. However, the risk assessment which is generally performed for any registration of chemical products does not yet cover corals for the most contaminants. Due to the sensitivity and importance of corals for the marine ecosystem, it is essential to include corals and develop a standard toxicity test protocol for corals as well. To generate reliable and comparable toxicity endpoints a standard protocol is unavoidable.

As the sensitivity of corals to chemical pollutants depends on species and life-stage, the risk assessment should consider larvae and coral nubbins (branch tips consisting of several polyps). For risk assessment with nubbins growth rates (e.g., of lengths and biomass), necrosis (loss of tissue) and bleaching should be assessed. Ranges for parameters like light intensity, pH, temperature, salinity, alkalinity, calcium-, nitrate- and phosphate content should be determined.

We screen potential coral species for ecotoxicological testing with focus on doubling of biomass during test period which is essential to evaluate effects on growth. Besides the property of growing, the species needs to be relevant, widespread, and sensitive and the coefficient of variation (CV) for the control group should be as low as possible.

**2.01.P-Mo033 Investigating Sublethal Impacts of Karenia brevis on Acropora cervicornis**

***Kaylie Costa***[1]*, Aileen Maldonado*[2] *and John A. Bowden*[1]*, (1) University of Florida, (2) Mote Marine Laboratory*

*Karenia brevis* blooms, known as “red tide”, have been documented off the coast of Florida since the 1840s, but increased bloom intensity and duration have been observed in recent years worldwide. This change may be attributed to excess nutrients entering waterways or other anthropological disturbances of coastal environments. *K. brevis* produces neurotoxins known as brevetoxins that have been linked to marine mammal, fish, bird, and invertebrate mortality, as well as sublethal impacts in humans including respiratory, immune, and neurological impairment. Although red tide events are most common in Southwest Florida, data from Mote Marine Laboratory shows that low to medium concentrations of red tide have been detected in the Florida Keys over the past 200 years. As these blooms continue to expand toward the Florida Reef Tract, following the Gulf of Mexico loop current, the impact of these toxins on coral health and reproduction remain unknown. The purpose of this project was to investigate the impacts of environmentally-relevant levels of red tide on *Acropora cervicornis*, an important Florida reef-building coral species widely used in the restoration setting. *A. cervicornis* fragments (n=30) were exposed to *K. brevis* for two weeks; treatments included a control group, 1,000 *K. brevis* cells/L, and 50,000 *K. brevis* cells/L. On the final day of exposure, fragments were flash frozen in liquid nitrogen. Untargeted mass spectrometric techniques were used for global profiling of alterations in both lipid and metabolite pathways caused by *K. brevis*. The results of this study will contribute to a greater understanding of the physiological impacts of *K. brevis* on coral health and highlight important areas for future research.

**2.01.P-Mo034 Evidence Base to Assess the Relationship Between Water Quality Stressors and Coral Reef Endpoints, in the Context of Interactions with Climate Effects**

***Roxolana Kashuba****, Jordan West and Nicholas Rosenau, U.S. Environmental Protection Agency*

Coral reef ecosystems are susceptible to a range of water quality stressors, such as nutrients, sediments, metals, and others, as well as particularly vulnerable to warming temperatures and ocean and coastal acidification processes associated with climate change. For water quality managers to make decisions about how to implement effective management actions, they need an understanding of what levels of water quality stressors are protective of corals given concurrent changes in climate stressors. Current marine water quality standards in U.S. coral reef jurisdictions are generally not crafted specifically for coral reef endpoints, and data gaps remain in understanding which stressor levels are protective of which coral reef endpoints, and whether they remain protective under a changing climate. To inform water quality management efforts, a systematic evidence mapping effort was implemented to create an evidence base of currently available peer-reviewed studies measuring (1) one or more water quality stressors, (2) one or more coral reef species or ecosystem endpoints, and (3) one or more adverse effects of the water quality stressor(s) on the coral reef endpoint(s). Studies were additionally identified by geographic location, study type (lab or field), and whether they assessed impacts of climate or management on coral reef health. Out of > 19,000 studies identified by systematic literature search, ~ 5,200 studies met the three inclusion criteria. More than half of the included studies assessed the effect of temperature, and a large proportion of the studies assessed impacts of pH and a variety of chemical stressors. There were as many field studies as lab studies, and twice as many studies assessing individual coral reef species as coral reef ecosystem endpoints. Less than 10% of studies reported water quality thresholds, while half of the studies reported a consideration of climate interactions. Mortality and bleaching were the most common adverse effects assessed; other effects included reproduction (egg size, fertilization, larval development), genetic markers, photosynthesis, growth, calcification, respiration, disease, tissue loss, enzyme activities, ecosystem community metrics, bacterial community diversity, and macroalgal cover. The result is a systematically compiled, synthesized and mapped evidence base that can be used to assess the type and availability of data for water quality management focused on coral reef protection.

**2.01.P-Mo035 Developing Standard Toxicity Assays in the Scleractinian Coral Acropora cervicornis**

*Dorothy-Ellen Abigail Renegar[1], Iain Davies[2] and **Carys Louise Mitchelmore**[3], (1) Nova Southeastern University, (2) PCPC (Personal Care Products Council), (3) University of Maryland Center for Environmental Science*

Coral reefs are some of the most biologically productive and economically important ecosystems in the world, with corals supporting significant diversity of marine life in tropical regions. Corals are directly or indirectly impacted by a combination of stressors including a diverse array of chemical contaminants. Furthermore, impacts of contaminants can be enhanced when combined with co-stressors, including UV radiation and those relating to climate change, such as ocean warming and acidification. However, clear assessment of the environmental risk of chemical contaminants to scleractinian corals is hampered as there are currently no US EPA or OECD standard test protocols for corals, and it is unclear how representative other standard test species (i.e. marine invertebrates and algal species) may be, given the complexity of the host-symbiont-holobiont system. Toxicity tests with corals have been conducted but the lack of a standard methodology has limited comparisons between studies and highlighted concerns on data reliability and quality. Building upon experiences conducting coral and other cnidarian toxicity tests with crude oil, polycyclic aromatic hydrocarbons, chemical dispersants, heavy metals, pesticides and UV filters we provide guidelines and discuss considerations moving forward on designing standard toxicity tests for scleractinian coral species. Factors include the relative sensitivity of individual coral species and/or life-stages to chemical contaminants, their availability and suitability to laboratory culture, specific test designs and replication requirements, inclusion of appropriate parameters for quality control (i.e. water quality, health assessments and appropriate negative and positive controls), timing of exposures, analytical verification, and finally the choice of appropriate biological endpoints for acute and chronic assessments to provide reliable and statistically appropriate measurements. The flow-through exposure system and methodology which was developed based on these principles was tested with two positive controls (copper and diuron) in acute and chronic exposures. Analytical verification in each replicate confirmed that reliable and reproducible concentrations in expected ranges (based on nominal dosing) were achieved, water quality parameters remained within guidelines, and coral health was maintained throughout the 21-day duration for chronic tests.

## 2.02 Advancing Aquatic Toxicity Test Methods: Considerations for Culturing, Testing and Data Analysis Test Methods

**2.02.T-01 Introductory Remarks - Advancing Aquatic Toxicity Test Methods: Considerations for Culturing, Testing and Data Analysis Test Methods**

***Natalie Love**, GEI Consultants, Inc.*

**2.02.T-02 Historical Test Variability for the Ceriodaphnia dubia Chronic Method: The California Experience**

***Darrin J. Greenstein**, Alvine Mehinto and Kenneth Schiff, Southern California Coastal Water Research Project (SCCWRP)*

The *Ceriodaphnia. dubia* survival and reproduction test is an established whole effluent toxicity test method commonly used in regulatory programs including the Toxicity Provisions recently adopted by the State of California. However, regulators and stakeholders recognize that some laboratories may need to improve their implementation of the *C. dubia* method to reduce intra-laboratory variability and increase comparability amongst laboratories. The present study aims to evaluate laboratory performance among those accredited by the state of California and investigate factors that can lead to test variability and decrease confidence in assessments of toxicity. To achieve the first part, data including control survival and reproduction, water quality, reference toxicant, and brood board information was collected from 17 accredited laboratories covering the previous 18 months to 3 years. Interviews were then conducted to obtain details of how each lab performed the test method. The data set is comprised of over 1000 control samples and 450 reference toxicant tests. The average number of neonates per female produced in controls ranged between approximately 17 and 35 between laboratories. The coefficient of variation of neonate production also showed sizeable differences both within and between labs. Statistical analysis did not reveal any specific test parameters, such as age of animals at the start of test, water quality or laboratory experience that explained the differences in reproduction results. Analysis of other testing parameters, such as food and dilution water production were confounded by the number of differences in procedures used by the laboratories; no two labs were using exactly the same set of methods. The next steps will involve testing of split samples to gain more insight into these differences.

**2.02.T-03 Assessment of Interlaboratory and Seasonality Impacts of Culturing Ceriodaphnia dubia with Light Emitting Diode (LED) Lights**

***Rebecca Cooper***[1], *Ashley Romero,*[2] *and Natalie Love*[2], *(1) Arkansas State University, (2) GEI Consultants, Inc.*

At the Society of Environmental Toxicology and Chemistry's 41st Annual Meeting in 2020, multiple presentations were given assessing the culturing and testing of various organisms under light emitting diode (LED) produced light and its growing prevalence in laboratories. As the world moves towards a more energy efficient and conservation-focused mindset, the lights that have historically been used in laboratories, primarily fluorescent and incandescent bulbs, are becoming harder to procure, leaving LED as the only option. While specific light intensity is recommended in the United States Environmental Protection Agency's (EPA) Methods for whole effluent toxicity (WET) testing, the medium to deliver the intensity is not. Studies presented by Arkansas State University and GEI Consultants, Inc. in 2020 demonstrated that while LED is clearly providing sufficient data quality for some species, additional work was needed to fully assess the impacts of LED for other WET species, specifically *Ceriodaphnia dubia*, under differing WET scenarios.

Additional culture data analysis and reference toxicant testing using the *C. dubia* species has been conducted by Arkansas State and GEI since 2020, and the complete datasets have been combined to assess, 1) whether LED lights provide consistent WET results across these two laboratories, 2) whether seasonal differences in species sensitivity to LED light are observed, and 3) whether culturing in LED impacts results in LED tests. All data analysis and study results will be summarized, and recommendations provided.

**2.02.T-04 Comparing the Toxicant Responses and Culturing Characteristics of Long-Term Laboratory-Reared and Field Populations of Ceriodaphnia dubia**

***Victoria Lydy***, *Rebecca Cooper and Jennifer Bouldin, Arkansas State University*

*Ceriodaphnia dubia* is an EPA-recommended organism used in Whole Effluent Toxicity (WET) testing for regulating wastewater discharge. Due to the ease of culturing and responsiveness to toxicants, it is also used in surface water and commercial chemical toxicity testing. For testing convenience, *C. dubia* is often cultured for extended periods in the laboratory with little knowledge of how it impacts subsequent generations of organisms. Extended laboratory rearing of *C. dubia* cultures could impact how they respond to stressors and decrease the accuracy of test results. This work investigated if a population of *C. dubia* cultured for an extended period in the laboratory was representative of field populations by comparing their sensitivities to toxicants and culturing characteristics. Comparative chronic toxicity tests with sodium chloride, the neonicotinoid insecticide, thiamethoxam, and mixtures of the two toxicants, were completed with a long-term laboratory-reared culture and three field populations of *C. dubia*. The results indicate that all three field populations are more sensitive to sodium chloride and less sensitive to thiamethoxam than the laboratory-reared *C. dubia* culture. Mixture test results indicate that the mixture had an additive effect on the survival and reproduction of *C. dubia* in all four populations. For culturing characteristics, the laboratory culture had longer body lengths, more neonates, and lower mortality rates on average than all three field populations of *C. dubia*. These differences support the idea that long-term laboratory-reared cultures might not be the best representative of aquatic invertebrates in regulatory toxicity testing and that alternative methods should potentially be pursued.

**2.02.T-05 Novel Approaches Isolate Contaminant Effects in Complex Mixtures Enabled by 3D Printing of Nature Inspired Designs**

***Alan Kennedy***[1], *Mark Ballentine*[1], *Lauren May*[1], *Travis Thornell*[2], *Chris Williams*[3] *and Michael Bortner*[4], *(1) U.S. Army Corps of Engineers, (2) U.S. Army Engineer Research and Development Center, (3) Virginia Tech, (4) Virginia Polytechnic Institute and State University*

Identifying the primary cause of toxicity in field-to-laboratory evaluations of discharges containing contaminant mixtures is challenging. Establishing defensible lines of evidence for toxicity drivers informs management decisions. Many field-collected samples containing persistent contaminants also contain elevated ammonia from anthropogenic and natural sources. This research investigates novel methods enabled by 3D printing (3DP) polymer-zeolite composites to isolate ammonia toxicity from the other contaminant effects without the treatment itself confounding bioassays. While zeolite columns remove ammonia, they cannot be deployed and recovered for *in situ* treatment and free particles harm organisms. Here, we compounded natural zeolite into the plant-derived polymer polylactic acid (PLA) to immobilize zeolite; this enables on-demand customization by 3DP high surface area, deployable, and retrievable structures that are inspired by natural stratified monoliths. Results determined that incorporating different zeolite loadings (8-32% w/w) in printed, submersible structures improved ammonia removal from bioassay media, with the 32% zeolite structure reducing ammonia below toxic levels within 24h treatment. Elutriate slurries generated from field-collected sediments containing metal, organic, and ammonia contamination were toxic to the toxicological model *Ceriodaphnia dubia* in standard 48h tests. However, after these waters were treated for 24h with the 3DP PLA-zeolite, the toxicity was completely removed. Since the concentrations of metals and organics were not appreciably altered following treatment, the submersible PLA-zeolite structures appeared to be specific for ammonia, which is desirable for ammonia-specific toxicity reduction evaluations. Follow-on experiments employed a Taguchi design of experiments to determine how different 3DP parameters (temperature, nozzle size, layer height, deposition speed) impacts the density and surface area of the printed structure. Printing faster at lower temperatures through larger nozzles resulted in lower density and greater porosity, leading to faster water absorption and greater ammonia adsorption. This provides faster treatment efficacy, which aids the conduct of future laboratory toxicity identification evaluations. This technology may be applicable to adsorption of other chemical classes by integration of different adsorbent powders into the printable polymer.

**2.02.T-06 Application of passive sampling and bioassays for assessment of the removal efficiency for bioavailable organic contaminants in wastewater treatment plants**

***Ratanang P.V. Mlaba***, *Tshepo J. Malefetse, Thabo T.I. Nkambule and Hlengilizwe Nyoni, University of South Africa*

Inadequately treated wastewater effluent contaminated with a wide range of pollutants pose a great concern and risk for the aquatic systems where it is discharged. In this study, the performance of wastewater treatment processes in removing bioavailable contaminants was evaluated using a combination of passive sampling and in vitro bioassays. The extracts from passive samplers (Chemcatcher, Polar organic chemical integrated sampler (POCIS), and Semipermeable membrane devices (SPMD)) were exposed to a battery of bioassays that targeted relevant modes of action such as non-specific toxicity, reactive toxicity, specific toxicity, adaptive stress response, and xenobiotic metabolism. High toxicity response was observed in the influent wastewater samples compared to the effluent samples. This study demonstrated that the combination of chemical analysis with batteries of bioassays covering a broad range of biological endpoints is suitable for assessing the performance/efficiency of wastewater treatment processes for the removal of bioavailable contaminants.

**2.02.T-07 Merging Alternative Toxicity Methods and Passive Sampling Towards Improved Stormwater Assessment**

*Marienne Colvin[1], **Nicholas T. Hayman**[1], Gunther Rosen[1], Chris Stransky[2], Charles Ginsberg[2] and Anthony Yamat[3], (1) Naval Information Warfare Center (NIWC) Pacific, (2) Wood Environment & Infrastructure Solutions, Inc., (3) NAVFAC SW*

Current standard practices in stormwater monitoring programs to assess environmental impacts of trace metals are not environmentally relevant to exposures experienced in the receiving environment. These practices include grab sampling from end of pipe (EOP) for chemistry measurements and whole-effluent toxicity (WET) testing using standard continuous exposure methods. Over the last several years, this project team has demonstrated two methodologies, pulsed exposure toxicity testing, and diffusive gradient in thin films (DGT) passive sampling, towards improving stormwater quality assessment. Both methodologies have been incorporated in San Diego Navy stormwater monitoring program since 2020, encompassing two stormwater seasons. Stormwater EOP samples were collected at industrial outfalls, and pulsed exposure testing was performed using purple sea urchin (*Strongylocenrotus purpuratus*) embryo-larval development tests. In addition to the standard 72-h exposure, urchin embryos were also exposed to the EOP sample for 26-h and a storm-specific duration (i.e. 6h of rainfall equates to 6hr of exposure to EOP sample). Following this shortened exposure, organisms were transferred to receiving water co-collected during sampling. DGTs were co-deployed in the laboratory toxicity tests for similar exposure durations. In addition, DGTs were deployed *in situ* within the receiving environment at the point of discharge for the stormwater for 72-h. Deployments *in situ* began just prior to the storm event and were in place during and after the storm event to capture ambient trace metal concentrations. DGT concentrations in concurrent field and laboratory exposures help to validate that pulse exposure toxicity responses in laboratory are more reflective of receiving water impact from storm water, measurement of which is a goal of NPDES Permits, than the current WET methodology. In addition, observed toxicity suggest that DGTs may be more relevant towards predicting toxicity than dissolved or total recoverable metal concentrations. Data from more recent storm events will be presented to further evaluate the efficacy of these methodologies to improve stormwater quality assessment, with the goal of enabling more accurate determination of exposure and risk in marine receiving waters related to stormwater runoff.

**2.02.T-08 Interpreting the Toxicity of Physically and Chemically Dispersed Oil: A Case Study with American Lobster Larvae (Homarus americanus)**

***Benjamin Patrick de Jourdan**[1], Danielle A. Philibert[1] and Joy Mcgrath[2], (1) Huntsman Marine Science Center, Canada, (2) ERM*

In oil toxicity studies, the presence of undissolved oil (present as microdroplets) creates obstacles in relating measured chemistry to observed biological effects. The chemical analysis of test solutions (e.g., WAFs and CEWAFs) reflect the total concentration of dissolved oil plus microdroplets, while it is generally the dissolved phase components that is driving the toxicity. Here we present a case study where data from a series of bioassays with American lobster larvae (*Homarus americanus*) exposed to dilutions of WAF and CEWAF were interpreted with different exposure metrics, including percent dilution, measured TPH fractions, measured parent PAH and alkyl-PAH, biomimetic extraction using solid-phase microextraction (BE-SPME), modelled toxic units (TUs), and dilution based toxic units as commonly used in whole effluent toxicity (WET) testing.

A two-step procedure for modeling whole oil dissolved toxic units was utilized to compute the dissolved components in each of the WAF and CEWAF dilutions. An empirical critical target lipid body burden based on the target lipid model framework was derived for lobster and applied in Petrotox to compute the TUs of each exposure solution. The WET testing toxic unit approach involved treating the WAF and CEWAF stocks as a whole effluent sample, thereby accounting for the collective hazard of all components within the test solution.

Each exposure metric is discussed in terms of pros and cons and their applicability and suitability to address different experimental objectives. Depending on the choice of exposure metric, the conclusions regarding the relative toxicity of physically and chemically dispersed oil differed. Exposure metrics which account for the bioavailable fraction, (e.g., TUs and BE-SPME), provide the best fit based on toxicity responses. Comparisons based on traditional exposure metrics (e.g., TPH or TPAH) can lead to incorrect conclusions regarding the relative aquatic hazards of physically and chemically dispersed oils. Expressing oil toxicity tests in terms of toxic units provides an opportunity to improve current practice by promoting comparison across studies, oils, loadings, weathering state and dispersants.

## 2.02.P Advancing Aquatic Toxicity Test Methods: Considerations for Culturing, Testing and Data Analysis Test Methods

### 2.02.P-Tu044 Lessons Learned in Refinement of Culturing and Toxicity Testing Methods for the Mayfly Neocloeon triangulifer

*Rebecca Dorman[1], **David J. Soucek**[1], Jeff A. Steevens[1] and Amy Dickinson[2], (1) U.S. Geological Survey, (2) University of Illinois, Urbana-Champaign*

Although insects occur in nearly all freshwater ecosystems, few sensitive insect models exist for use in determining the toxicity of contaminants. Recent literature has demonstrated the sensitivity of mayflies to environmental contaminants in both laboratory and field assessments, and the parthenogenetic mayfly *Neocloeon triangulifer* has been shown to be amenable to continuous culture in the laboratory. Methods for this species in both acute and chronic toxicity studies were published nearly a decade ago, but within that time frame, we have continued to refine our knowledge of the species' sensitivity and its needs in a culturing and testing context. In terms of sensitivity, we have learned that *N. triangulifer* is among the most sensitive of species to major ions, metals, and organic chemicals like solvents, perfluoroalkyl substances, and neonicotinoid pesticides. However, while it is useful to have a sensitive insect model for toxicity testing, it is important to be sure we are observing intrinsic sensitivity, not sensitivity due to culturing unhealthy or stressed animals. This is complicated for this species because we culture the animal and its diet. We have improved our culture and testing success by improving the quality of their diet. This includes how long diatom biofilms are of sufficient quality; when the diatom cultures need further nutrient supplementation; how diatom biofilm age can influence mayfly sensitivity in acute tests; and how to recognize a potentially low-quality diatom scraping. For example, we conducted three concurrent acute reference toxicity tests with sodium chloride using the same batches of treatment waters and the same cohort of <24-h-old mayflies. The experimental treatment for this study was varying phase of growth for diatom scrapings, manipulated by different age and/or nutrient supplementation levels. The 96-h acute values for these tests varied by a factor of two, indicating diet quality strongly affects *N. triangulifer* sensitivity.

### 2.02.P-Tu047 Assessing the Impacts of Two Coal Ash- Associated Trace Metals on the Viability, Locomotor Behavior, and Embryonic Development of the Freshwater Snail Planorbella Duryi

***Talia Tanner**, Leanna Giancarlo, Ben Odhiambo Kisila and Tyler Edward Frankel, University of Mary Washington*

Coal ash (CA) has been shown to contain several neurotoxic trace metals which are able to escape into local aquatic environments via accidental release, leaching from lined or unlined repositories, and permitted discharge. While previous studies have mainly focused on the impacts of these contaminants on freshwater teleosts, little is known about their effects on non-model aquatic invertebrate species. As such, this study assessed the exposure effects of two CA-associated trace metals, cadmium and arsenic, on the viability, locomotor behavior, and embryonic development of the Seminole ramshorn snail (*Planorbella duryi*). Exposure treatments were prepared at 0, 0.01, 0.1, 1, or 10 mg/L for each contaminant using either $CdCl_2$ or $Na_2HAsO_4$ and concentrations confirmed using ICP-OES. Individual adult *P. duryi* were placed into separate 400mL beakers and exposed for 10 days, during which viability was assessed every 24hrs. Locomotor behaviors were recorded on days 1, 5, and 10 and analyzed using automated behavior software (ToxTrac v2.97) to assess differences in average speed, acceleration, distance travelled, and time spent immobile. Newly laid (<6hrs old) embryonic clutches were obtained from a breeding colony. Individual embryos were then isolated from each clutch, placed into separate glass petri dishes containing a given treatment, and checked daily for embryonic development stage and hatching success. While this study is currently ongoing, we expect to find dose-dependent decreases in locomotor behavior and delays in development for both trace metals, with viability being the least sensitive endpoint of those tested. Our results will provide novel information regarding these contaminants which will enhance our understanding of their impacts on invertebrate populations that inhabit CA-contaminated waterways.

**2.02.P-Tu048 Water quality screening: A case study of the Diep River (Milnerton), Western Cape, South Africa**

***Asmat Begum Khan**, Omoniyi Kolawole Pereao and Beatrice Opeolu, Cape Peninsula University of Technology, South Africa*

Rivers serve as habitats for several aquatic organisms, and they play important roles in the water cycle as well as other ecosystem functions. The presence of chemicals in the environment remains a global challenge that requires monitoring and mitigation. The complementary use of chemical analyses with biotests provides more robust information about ecosystems' response to pollution events. The Diep River runs through the City of Cape Town across neighbourhoods with different land use types into the ocean. In this study, water quality of the Diep River was assessed. Surface water samples were analysed for physico-chemical parameters and bioassays. Three test organisms, each representing a trophic level, were exposed to the river water samples. The organisms used were *Raphidocelis subcapitata* (microalgae), *Daphnia magna* (crustacean), and *Tetrahymena thermophila* (protozoan). The pH values ranged between 7,73 and 9,83; dissolved oxygen was between 2,4 and 11,8 mg/L; conductivity -676 and 20000,0 m/s; and temperature values ranged between 15,8 and 23,2 ºC. The results revealed that the Diep River water physico-chemical parameters were within the South African Water Quality standards with a few exceptions. The ecotoxicological approach used can add value to hazard and risk assessment of the river and contribute to the management of water quality along the Diep River.

**2.02.V Advancing Aquatic Toxicity Test Methods: Considerations for Culturing, Testing and Data Analysis Test Methods**

**2.02.V-01 The fish early-life stage sublethal toxicity syndrome - A high-dose baseline toxicity response**

***Jim Meador**, National Oceanic and Atmospheric Administration (NOAA)*

A large number of toxicity studies report abnormalities in early life-stage (ELS) fish that are described as a sublethal toxicity syndrome and generally include a reduced heart rate, edemas (yolk sac and cardiac), and a variety of morphological abnormalities. This syndrome of effects is very common and not diagnostic for any chemical or class of chemicals. This sublethal toxicity syndrome is mostly observed at high exposure concentrations and appears to be a baseline, non-specific toxicity response; however, it can also occur at low doses by specific action. Toxicity metrics for this syndrome generally occur at concentrations just below those causing mortality and have been reported for a large number of diverse chemicals. Predictions based on tissue concentrations or quantitative-structure activity relationship (QSAR) models support the designation of baseline toxicity for many of the tested chemicals, which is confirmed by observed values. Given the sheer number of disparate chemicals causing this syndrome and correlation with QSAR derived partitioning; the only logical conclusion for these high-dose responses is baseline toxicity by nonspecific action and not a lock and key type receptor response. It is important to recognize that many chemicals can act both as baseline toxicants and specific acting toxicants likely via receptor interaction and it is not possible to predict those threshold doses from baseline toxicity. We should search out these specific low-dose responses for ecological risk assessment and not rely on high-concentration toxicity responses to guide environmental protection. The goal for toxicity assessment should not be to characterize toxic responses at baseline toxicity concentrations, but to evaluate chemicals for their most toxic potential.

**2.02.V-02 Multigenerational Effects of Benzotriazole in Daphnia Magna at Molecular and Physiological Levels**

***Hyungjoon Im***, *Macha Fulgence Jacob, Jiyeon Kang and Jeong-Eun Oh, Pusan National University, Korea, Republic of (South)*

Due to its widespread and intensive use as corrosion inhibitor, benzotriazole is ubiquitously detected in aquatic environment. The present study aimed to investigate multigenerational and transgenerational effects of benzotriazole in molecular and physiological levels using freshwater zooplankton *Daphnia magna*. The results indicate that exposure to sublethal concentrations (15 and 30 mg/L) of benzotriazole cause transgenerational effects on *D. magna* with molecular and physiological level effects even in descendants that have never been exposed to benzotriazole. Especially, significant alterations in expression of Cyp, GST, Vtg1 and Hb genes and neonate size were observed in the unexposed F3 generation, confirming the distinct transgenerational effects of benzotriazole. Furthermore, it was demonstrated that the continuous multigenerational exposure to environmental concentration (4.3 μg/L) of benzotriazole leads to molecular level effects but no physiological level effects in the subsequent generations. The findings of this study will contribute to a better understanding of the multigenerational effects of toxic chemicals on aquatic organisms.

**2.03.P Assessing Contaminant Effects in Ecosystems with Multiple Stressors**

**2.03.P-Mo036 A History of Ancestral Bisphenol A Exposure Can Be A Confounding Factor for the Second Hit by Environmental Levels of Perfluorooctane Sulfonic Acid**

***Seraiah Tate Coe***, *Sourav Chakraborty and Ramji K. Bhandari, University of North Carolina Greensboro*

Studies suggest that bisphenol A (BPA) contamination of the environment began seven decades ago. Ancestral BPA exposure-specific epimutations, linked to fertility and liver health, have been established in the germline genome, making its passage to subsequent generations possible. Therefore, the current generation now harbors these ancestral epimutations while being exposed to novel contemporary environmental chemicals of emerging concern (CECs), such as perfluorooctane sulfonic acid (PFOS). PFOS has well documented oleophobic and hydrophobic properties which led to its use in numerous commonplace items. This ubiquitous use resulted in environmental contamination and subsequent bioaccumulation in both animals and humans. Links between PFOS exposure and defects that affect reproduction, early development, and liver health have been established. However, it is not clearly understood if individuals with a history of ancestral BPA exposure have altered sensitivity to exposure to CECs, particularly PFOS. This study will address this issue using medaka fish (*Orizias latipes*) as an animal model. Two groups of medaka were utilized, one group whose ancestors were exposed to BPA five generations ago and another group with no history of ancestral BPA exposure. Exposure to five concentrations of PFOS (0.002 mg/L, 0.02 mg/L, 0.2 mg/L, 2 mg/L, 20 mg/L) occurred continuously during key cell differentiation phases in procedure one. In procedure two, adults were exposed to four concentrations of PFOS (0.002 mg/L, 0.02 mg/L, 0.2 mg/L, 2 mg/L) for 21 days. The results of procedure one suggest that the individuals with a history of ancestral BPA exposure are at an increased risk of developing teratogenic phenotypes when exposed to environmentally relevant concentrations of PFOS during early development. In procedure two, we anticipate that following exposure to environmentally relevant concentrations of PFOS, adult fish with a history of ancestral BPA exposure will display decreased egg production and fertilization rate as well as increased lipid content in the liver. These findings are essential in establishing the previously unexplored relationship between ancestral BPA exposure and contemporary exposure to CECs. This relationship would define a new variable in toxicological research and could be utilized to inform future perfluoroalkyl substance regulations, while additionally providing an important variable in the study of environmental injustice.

**2.03.P-Mo037 Quantification of heavy metals in soils and food crops within the vicinity of an Integrated Agro Industry and the associated ecological and potential human health risks**

*Dorris Ogechukwu Adibe, Janefrances Ngozi Ihedioha, Hillary Onyeka Abugu, Cynthia Nkoli Ibeto and* ***Nwachukwu Romanus Ekere****, University of Nigeria, Nigeria*

The contamination by potentially toxic heavy metals in soils and crops which in turn reduces the quality of food has been a serious health threat to consumers. Soils and crops within the vicinity of an integrated agro industry were analyzed for Chromium, Cadmium, Lead, Copper, Nickel and Zinc using Flame Atomic Absorption Spectrophotometer and their potential human health risks and ecological risks were assessed. The results showed mean concentration ranges (ppm) of the heavy metals in crop during dry season as follows: Cr = (0.1556 to 0.4278), Cd = (0.0238 to 0.0833), Pb = (0.2500 to 0.2600), Cu = (0.0216 to 0.1357), Ni = (0.1030 to 0.3761), Zn = (0.0116 to 0.1472). The mean concentration ranges (ppm) of the heavy metals in crop during wet season as follows: Cr = (N.D to 0.3500), Cd = (N.D to 0.0595), Pb = (N.D to 0.1986), Cu = (0.0215 to 0.0862), Ni = (0.1026 to 0.1118), Zn = (0.0089 to 0.0721). The mean concentration ranges (ppm) of the heavy metals in soil during dry season are: Cr = (0.1182 to0.3889), Cd= (0.0595 to 0.1310), Pb = (0.2600 to 0.8333), Cu = (0.0331 to 0.1832), Ni = (0.2067 to 0.7863), zinc = (0.1155 to 1.7547). The mean concentration ranges (ppm) of the heavy metals in soil during wet season are: Cr = N.D to 0.1556,Cd =0.0238 to 0.0476,Pb = 0.2979 to 0.3972, Cu = 0.0215 to 0.0862, Ni = 0.1031 to 0.4445, and Zn= 0.0116 to 0.1010. The non-carcinogenic human health risk assessment of cassava tubers (*Manihot esculenta)* showed the health risk index are:farm 1, Pb = 2.597 for adults and 9.326 for children in dry and 1.980 for adults and 7.126 for children in wet season, Cd = 0.341, Cr = 0.447, while Cu, Zn and Ni showed much less health risk values;farm 2, Cr =1.228 and 1.000 for children in dry and wet seasons respectively), other metals showed much less human health risk values; on farm 3, Pb = 2.497 for adults and 8.972 for children in dry season, cadmium =0.683 and 0.854 for children in dry and wet season respectively and 0.190 and 0.238 for adults in dry and wet season respectively, while the other metals have lower health risk values. The carcinogenic human health risk assessment showed that all metals assayed had incremental life-time cancer risk (ILCR) value of $10^{-3}$ - $10^{-6}$ for both children and adults on all the farms. The contamination factor (Cf), degree of contamination (Cd), pollution load index (PLI), and transfer factor (Tf) were used to assess the ecological risk and all were found to be below their threshold limits.

**2.03.P-Mo038 Assessing Ceriodaphnia dubia and Chironomus dilutus bioassays of the Middle White River subwatersheds, Arkansas, USA**

***Rainee DeRoin*** *and Jennifer Bouldin, Arkansas State University*

Surface water is essential for domestic, agricultural, and industrial uses and is under increasing threats of pollution from industrial and agricultural sources. In Arkansas, the Middle White River Watershed is on the 303 (d) list of impaired waters as established through the Clean Water Act. The 303 (d) list was established as a way for the EPA and Congress to monitor water quality and allow states to verify quality standards for specific waterways intended uses are met. The Middle White River is listed as impaired due to high turbidity and total suspended solids as well as low dissolved oxygen and presence of pathogens. The watershed is used year-round for recreational and commercial activities and is primarily forested with pastures dominating agricultural use. Row crop land cover dramatically increased from 1% in 2004 to 5.4% in 2006 in the Middle White River Watershed. The shift in land use around the watershed is a potential source of impairment. Many subwatersheds located in the Middle White River are lacking sufficient data for water quality assessment. In order to see how the subwatersheds have been impacted bioassays were used to measure the survival and reproduction of aquatic organisms. The whole effluent test (WET) was conducted with *Ceriodaphnia dubia* in a seven-day chronic test collected during each season from Fall 2021 to Summer 2022. Sediment was also collected seasonally for acute toxicity testing used *Chironomus dilutus* in a ten-day test. The organisms in both bioassays have shown some adverse effects throughout the seasons and sample sites. This leads to the possibility that there are potential contaminants within the subwatersheds that would contribute to the Middle White River to continue being on the 303(d) list of Arkansas for impairments.

**2.03.P-Mo039 Transcriptomic Effects of Wastewater Treatment Plant Discharge to the Brain of in situ Exposed Juvenile Atlantic Cod (Gadus morhua) in Norway**

***Jason Tyler Magnuson**, Magne Sydnes and Daniela Maria Pampanin, University of Stavanger, Norway*

The release of contaminants of emerging concern (CECs) into the aquatic environment from runoff, aquaculture, and effluent from wastewater treatment plants (WWTPs) may threaten the health of aquatic organisms. To better understand the input and movement of CECs from a predominant, regional WWTP into the Stavanger fjord and potential toxicity to an environmentally and economically important fish species, a Dose related Risk and Effect Assessment Model (DREAM) was used to track the dispersion of complex chemical mixtures from the discharge plume. The model consisted of ocean currents, discharge rate, and physical, chemical, and toxicological properties of CECs within the system as prediction input factors. This model was used to select locations within the fjord to monitor the movement of the discharge and assess potential toxicity to Atlantic cod (*Gadus morhua*). Fish were caged at 3 sites: the WWTP discharge (IVAR), an aquaculture facility (Aqua), and a reference site (Kvistøy), for four weeks. Brains were dissected from male and female individuals and transcriptomic profiling assessed. The top predicted canonical pathways in male Atlantic cod caged at IVAR were involved in estrogen, glucocorticoid, and aryl hydrocarbon receptor signaling pathways, with disease and functions related to impaired growth of genital organs and neural cell proliferation, as determined through pathway analysis software. PPARα/RXRα activation, neuroinflammation signaling pathway, and glucocorticoid receptor signaling were among the top predicted canonical pathways in male fish caged at Aqua, with disease and functions involved in the transport of molecules and central nervous system inflammation. Top predicted canonical pathways in female Atlantic cod caged at IVAR were involved in CREB signaling in neurons and axonal guidance signaling, with motor dysfunction/movement disorder and hyperactivity among the top disease and functions altered. Synaptogenesis signaling pathway and glucocorticoid receptor signaling were among the top predicted canonical pathways in female fish caged at Aqua, with disease and functions involved in the formation of intermediate filaments and dissociation of neurofilaments. These data indicate that CECs in the proximity of the WWTP discharge are dispersed from the plume into rearing habitats for native fish species and compounds present in the discharge have multiple targets within the brain that impair motor neuron function and induce estrogenic effects.

**2.03.P-Mo040 Whole-ecosystem Characterization of Metal Exposure in a Natural Tundra Wetland Receiving Wastewater**

***Bronte McPhedran**[1], Mark L. Hanson[2] and Heidi Swanson[1], (1) University of Waterloo, Canada, (2) University of Manitoba, Canada*

Variability in metal concentrations over the lifetime of a fish cannot be elucidated using muscle, or any other organ that is metabolically active, but may be reconstructed using metabolically inert tissues that continuously accrete, such as otoliths. Time-resolved analysis is useful in environments that lack baseline data or where challenges preclude regular monitoring, such as in the Arctic. Wastewater in the Arctic is primarily treated via natural tundra wastewater wetlands that rely on biochemical processes and dilution for effective treatment. Although favoured due to minimal maintenance and infrastructure, performance is constrained by the region’s extreme climates and increasing human activity. We aim to understand past and present metal accumulation in fish downstream of the wastewater treatment lagoon in Baker Lake, Nunavut, Canada. We used the natural tundra wastewater wetland in Baker Lake, Nunavut, as a case study to 1) investigate metabolically inert fish otoliths as a monitoring and assessment tool for metal exposure, and to 2) characterize routes of metal exposure into different fish tissues (i.e., otoliths, muscle, and liver) from upstream (n=1), wastewater-affected (n=3), and reference (n=2) lakes. Biological, chemical, and physical lake characteristics were sampled in July and August of 2019 and 2021. Parameters included large- and small-bodied fish, benthic macro-invertebrates, zooplankton, sediment, and water. Results to date depict a distinct separation of variables based on a principal component analysis of otolith marginal edge metal concentrations among sites. Elements that contribute the greatest to differences in marginal edge metal concentrations are manganese, zinc, and selenium. Distinct variables among impacted and reference lakes suggests a possible wastewater metal "signature" in the most recent year of fish growth (i.e., the otolith marginal edge). This study will provide valuable information on the current state of fish health in the Baker Lake wastewater treatment system and help advance otolith microchemistry as an accessory monitoring technique in wastewater and in Arctic environments.

**2.03.P-Mo041 Nutrient Activity in Headwaters of the Buffalo River**

***Patrick Dyer** and Jennifer Bouldin, Arkansas State University*

Nonpoint source pollution of U.S. waters may be driven by specific pairings of agricultural practices and meteorological conditions. Monitoring upstream sediment and nutrient loads is necessary in managing the eutrophication occurring in the Gulf of Mexico and elsewhere. Water quality collected weekly at eight locations along four headwater streams on the Buffalo River in Arkansas included dissolved and total nutrients as well as total suspended solids (Nov. 2019 – Jan. 2022). The Buffalo River is an integral part of the Mississippi River Watershed and flows 250 kilometers across the state draining 3500 square kilometers. The small headwater streams being sampled are a primary drainage to the Gulf of Mexico, and their ability to store and release nutrients is critical to downstream health. These small streams reflect a local scale and are studied as microcosms of a larger watershed. During baseflow conditions the studied tributaries are categorized as "least disturbed" systems and carry less sediment than more disturbed reaches alongside rowcrop or developed land, but certain studies suggest it is relatively few large-scale precipitation events that drive the erosion and resuspension of nutrients driving seasonal hypoxic events. While the lack of significant flow alteration is often commended; development, in particular the conversion of forest to pasture, may trouble the Buffalo Rivers future.

**2.03.P-Mo042 Factors affecting photo-induced thin oil sheen toxicity in a model early life stage (ELS) fish (Danio rerio)**

***Rachel R. Leads**[1], Corey Green[1], Michelle Krasnec[2], Jeffrey Morris[2] and Aaron Roberts[1], (1) University of North Texas, (2) Abt Associates*

In the aquatic environment, organisms may be exposed to crude oil in several different ways including dietary exposure, uptake of dissolved constituents, contact with oil droplets in the water column, or contact with thin oil sheens concentrated on the surface of the water. Thin sheens or slicks are thin layers of oil (~1 µm thick) that float on the surface of the water and are formed during oil transport and weathering processes in the environment. For early life stage (ELS) and planktonic organisms that occupy surface waters, thin sheens may be an important and under-studied route of oil exposure. In addition, ultraviolet (UV) light exposure in surface waters may enhance the toxicity of thin sheens to aquatic organisms due to photo-induced toxicity of oil constituents. Laboratory tests indicate that thin oil sheen and UV co-exposure is acutely toxic to several ELS fish and invertebrate species. However, toxicity data are currently limited and exposure mechanisms (e.g., physical contact with the sheen or exposure to underlying water) require further elucidation. To better understand these exposure mechanisms, the present study investigated thin sheen toxicity and UV co-exposure using ELS (4-96 hours post-fertilization) zebrafish (*Danio rerio*) as a model organism. Thin sheens and UV co-exposure significantly decreased survival of ELS zebrafish ($p<0.0001$). At 96 h, percent survival (mean ± SE) of ELS zebrafish exposed to thin sheens in the absence and presence of UV was 90.0% ± 10.0 and 10.0% ± 5.0, respectively, indicating an effect of photo-induced toxicity. With thin sheen and UV co-exposure, percent survival also varied significantly with the volume of water underlying the sheen. Percent survival (mean ± SE) of ELS zebrafish co-exposed to thin sheens and UV in 200 mL of water (71.0% ± 12.5) was significantly lower than those exposed in 400 mL (92.0% ± 2.0) or 800 mL (95.0% ± 2.2) of water ($p=0.0003$), suggesting that the concentration of dissolved constituents underlying the sheen influence exposure and toxicity. Together, these results provide novel data on the toxicity of thin sheens and UV to a model fish species as well as new insights into the mechanisms of thin sheen exposure in aquatic organisms.

**2.03.P-Mo045 Multi-Stressor Effects of Temperature, Salinity, and Ultraviolet Light on PFOS Toxicity in Larval Estuarine Organisms**

***Katy W. Chung****, Peter B. Key and Marie E. DeLorenzo, National Oceanic and Atmospheric Administration (NOAA)*

Perfluorooctane sulfonate (PFOS) is a persistent contaminant that has been found globally within the environment. Key data gaps exist in the toxicity of PFOS to marine organisms, especially estuarine species that are crucial to the food web: fish, shrimp, and molluscs. This study aims to develop toxicity thresholds for four larval estuarine species: grass shrimp (*Palaemon pugio*), mysids (*Americamysis bahia*), sheepshead minnows (*Cyprinodon variegatus*), and eastern mud snails (*Tritia obsoleta*). Abiotic stressors (salinity, temperature, and ultraviolet light) were combined with PFOS exposure to determine if these climate variables alter PFOS toxicity. Definitive 96-h acute testing with larval *C. variegatus* resulted in LC50 estimates of between 0.85 (32°C and 10ppt) to 1.85 mg/L (25°C and 20ppt) for PFOS. Definitive 96-h acute testing with PFOS resulted in LC50 estimates of between 0.72 (32°C and 10ppt) to 5.04 mg/L (25°C and 20ppt) for larval *P. pugio*. PFOS toxicity was not affected by the addition of UV light. These two species would be protected by the proposed EPA salt water benchmark for PFOS (0.55 mg/L), although not necessarily under high temperature and low salinity conditions. PFOS testing with abiotic stressors is underway for mysids and snails.

**2.03.P-Mo047 The influence of annual variability on interpretation of whole organism characteristics in Trout-perch (Percopsis omiscomaycus) to inform design of long term monitoring programs**

***Stephanie Marshall***[1]*, Kelly Munkittrick*[1]*, Mark McMaster*[2]*, Patricija Marjan*[1] *and Thomas Clark*[2]*, (1) University of Calgary, Canada, (2) Environment and Climate Change Canada*

Environmental Effects Monitoring (EEM) is a Canadian cyclical, national mandatory requirement for several industrial sectors that tests whether facilities in compliance with their discharge regulations are associated with impacts on fish, fish habitat or fish use. EEM utilizes whole organism characteristics in fish such as body size, growth rates, organ sizes, and fecundity to establish whether exposed fish are different than reference fish. A variety of studies have shown that the optimal timing for measuring fish characteristics varies with reproductive strategy and season. In some species, relative liver size can vary as much as 5-fold over the annual cycle, and female relative gonad size can vary 40-fold. Trout-perch (*Percopsis omiscomaycus*) is a widely distributed small-bodied fish species that is becoming more commonly used in environmental monitoring in Canada. As trout-perch become more widely used, information on annual variability, as well as local and regional variability will be critical for designing sensitive monitoring programs. We sampled trout-perch over an annual cycle from July 2021 to July 2022 during ice-free months and measured size, age, condition, relative organ size, sex steroid hormone production, and contaminant levels. Female liversomatic index (LSI) was seen to decline to an average of 1.2% in August of 2021. In September 2021 female trout-perch were seen to have their smallest gonadosomatic index (GSI) average of 1.8% and largest LSI average of 4.9%. Since beginning monthly sampling in 2022 female GSI has reached a maximum average of 14% in late May, just prior to spawning. Considering that the pre-spawning time is a sensitive period in the annual life cycle, and trout-perch are showing maximum development, the most optimal time to sample trout-perch should be in mid-May prior to spawning.

**2.03.P-Mo048 Combined Effects of Cadmium and Microplastics on the Freshwater Leech, Nephelopsis obscura**

***Lauren Zink*** *and Gregory Pyle, University of Lethbridge, Canada*

Both microplastics and metals are contaminants detected in many freshwater systems globally. The interactions of microplastics with other contaminants including cadmium, a toxic metal, poses potential threats to the health of aquatic organisms including *Nephelopsis obscura*, a predatory leech species that is widespread across Alberta and serves as an important food source for many species. To assess the combined toxicity of cadmium, microplastics, and their mixture, leeches were exposed to the contaminant(s) for four weeks. Leeches show a high survivability following chronic exposure in cadmium-contaminated water at and above local water quality guideline limits established for cadmium. Leeches exposed to microplastics and cadmium-microplastic mixtures lost significantly more mass over the course of the exposure than those exposed to cadmium only or not exposed to contaminants. Further, leeches exposed to microplastic only and cadmium-microplastic mixture secreted more mucus than those exposed to cadmium only or not exposed to contaminants. Behavioural assays following exposure were conducted to assess foraging behaviour, as leeches primarily scavenge. Leeches that were exposed to cadmium and cadmium-microplastic mixtures were not able to locate food as efficiently as leeches exposed to microplastics only or not exposed to contaminants. This study highlights the importance of assessing sensitive sub-lethal endpoints that affect individual and population health. This work suggests that cadmium and microplastics affect the leech in different ways, but that co-contamination of cadmium and microplastics in a system can result in greater deficits to the leech than either contaminant present alone.

**2.03.P-Mo049 Cumulative effects of chemical mixtures and temperature on Daphnia magna**

***Sophie Steigerwald**, Gastón Alurralde, Sebastian Abel, Ann-Kristin Eriksson-Wiklund, Anna Sobek and Elena Gorokhova, Stockholm University, Sweden*

In the environment, chemical mixtures act in concert with additional stressors, such as temperature and other physicochemical factors, which may influence the exposure effects. However, assessing the toxicity of chemical mixtures with low concentrations of each component is a challenging task, particularly, when the interactive effects of the chemical exposure with other stressors are of interest. Here, we used a novel combination of the chemical activity concept and degree-days method to evaluate interactions between the chemical exposure and thermal stress in *Daphnia magna*. The chemical activity approach simplifies toxicity assessment for a mixture of neutral organic contaminants, with baseline toxicity (narcosis) occurring between 0.01 and 0.1. We hypothesized that in the acute toxicity test with a chemical mixture in this activity range and under different temperatures: (1) daphnid mortality follows a dose-dependent response to the chemical activity, (2) lower median lethal activity (La50) occurs at a higher temperature if the exposure time is defined in calendar days because of the temperature effects on the metabolic rate and time-to-toxicity, and (3) there is no temperature effect when the exposure time is defined in degree-days representing the physiological time of the daphnids at different temperatures. The mixture of four polycyclic aromatic hydrocarbons (acenaphthene, fluorene, fluoranthene, and phenanthrene) was tested using a passive dosing system and three temperature regimes (20, 23, and 25˚C). The endpoints include survival and weight loss during the exposure to estimate the La50 values and metabolic effects in concert. The experimental results will serve to develop recommendations for the implementation of the chemical activity approach in the hazard assessment of organic contaminants in aquatic environments with variable thermal regimes.

**2.03.P-Mo050 In Vitro Mixture Toxicity to Endocrine Disrupting Chemical Combinations Used in Household Chemical Products**

***Inhye Lee** and Kyunghee Ji, Yongin University, Korea, Republic of (South)*

Consumers are exposed to various endocrine disrupting chemicals (EDCs) through the use of products in their daily life. In this study, frequent combinations of EDCs contained in cleaners, synthetic detergents, fabric softeners, air fresheners, and deodorant agents were identified using association rule mining. Then, one case of this combination was selected (hexyl cinnamaldehyde (HPA) and 2-(4-tertbutylbenzyl)propionaldehyde (2PA)), and the effects of mixture on steroidogenesis of H295R cells was investigated. There are 276 EDCs in the target products (n=5,984), and these substances were mainly used as fragrances or preservatives. When HCA and 2PA were individually exposed, the production of 17β-estradiol (E2) and testosterone (T) in H295R cells were significantly increased. However, when these substances were mixed in a ratio of 1:1, E2 production was significantly reduced. These results suggest that antiestrogenic properties that were not observed in individual substances may appear when these two substances are mixed. Acknowledgement: This study was supported by Korea Ministry of Environment (project number 1485017189).

**2.03.P-Mo051 Effects of Nutrients on Mercury Bioaccumulation at the Base of the Coastal Food Web**
***Anika Agrawal**, Rob Mason and Jessica Brandt, University of Connecticut*
Coastal systems are at risk of rapid environmental change and are sensitive to anthropogenic stressors such as metal pollution. Specifically, mercury (Hg) and methylmercury (MeHg) concentrations have increased in coastal areas and seafood resources. This has led to concerns about MeHg bioaccumulation in shellfish and finfish as seafood is the primary route of MeHg exposure to human consumers in the United States, and exposure to elevated MeHg concentrations has been associated with a suite of adverse health impacts including neurotoxicity and teratogenicity.

Our work is focused in the Long Island Sound where Hg concentrations are increasing in bivalves. Nutrient availability is thought to mediate Hg bioaccumulation and studies have shown that selenium (Se), which is increasing in the LIS, can act as a nutrient mediator of Hg biogeochemistry as well. However, Se's role as a nutrient at the base of the food web has been largely unstudied, despite basal food web interactions greatly influencing bioaccumulation and biomagnification in coastal food webs. To address these gaps in understanding, we are investigating the influence of macro- and micronutrients on the spatial and temporal dynamics of mercury bioaccumulation to phytoplankton and trophic transfer to juvenile and adult oysters. This project involves a two-year field study involving monthly field collections for Hg and nutrient concentrations in seawater, phytoplankton, and oysters of two age classes along a longitudinal gradient along the Connecticut portion of the LIS. We will assess the spatio-temporal relationships between nutrient concentrations, dissolved organic carbon, temperature, salinity, and Hg/MeHg and whether variation in these parameters explain changes in Hg/MeHg concentrations in phytoplankton to oysters. We anticipate that our results will inform how nutrient concentrations affect Hg cycling, leading to a better understanding of nutrient/Hg dynamics in coastal food webs. This poster will highlight the results of the year 1 (2022) sampling effort.

**2.03.P-Mo053 Evaluating Sensitivity and Variability of the Floating Percentile Model for Sediment Quality Benchmark Development**
***Brian G Church**, Claire Detering and John Toll, Windward Environmental LLC*
The Floating Percentile Model (FPM) generates sediment quality benchmarks (SQBs) using paired sediment chemistry and toxicity data. Recently, we independently revised the Floating Percentile Model in R to expand and improve on the existing Excel-based tool. Washington State's sediment management standards are based on the FPM, and the FPM was used for the Portland Harbor Baseline Ecological Risk Assessment. The R-based tool allowed us to understand the FPM's benefits and limitations. Simulations were used to evaluate variability in FPM outputs from resampling, and empirical and simulated data were used to evaluate sensitivities of FPM parameters. The FPM parameters that were evaluated included categorical variables, such as whether to assume normality and equal variance of concentrations, and numeric variables, such as the alpha level for hypothesis testing, precision of SQB calculations, and the false negative limit, which defines the conservatism of SQBs.

The overarching finding of our analyses was that the chemical selection step, which occurs at the beginning of the FPM algorithm, is a major driver of both output variability and sensitivity of parameters. Larger sample sizes of simulated data resulted in lower SQB variability, and there was a strong correlation between sample size, SQB variability, and the frequency at which chemicals were selected by the FPM. FPM outputs were most sensitive to the alpha level parameter and the false negative limit, both of which reflect policy decisions regarding certainty and conservatism. Parameter sensitivities were much higher for the empirical dataset than the simulated datasets (on average) due to the weaker distinction between chemical concentrations among toxic and non-toxic samples in our real-world example.

The R-based FPM tool is being published with tools to help users evaluate chemical selection and to optimize parameter inputs. General uncertainties associated with SQBs that are not necessarily addressed by the FPM include using bulk sediment chemistry without considering bioavailability and using toxicity data with high replicate variability. Each FPM SQB set, being a predictive tool rather than mechanistically defined, is suitable for screening-level purposes, not for risk-based cleanup levels. Still, the FPM represents a conceptual evolution for SQB development, in that it provides for data quality control, flexibility, and site-specificity, and it can address multiple chemicals at the same time.

**2.03.P-Mo055 Investigating Chemicals of Emerging Concern in Aquatic Ecosystems of Ecologically Diverse King County, Washington: Towards More Strategic Study Design**

***Jennifer Sampson White*** *and Jenee Colton, King County Department of Natural Resources and Parks*

At nearly 6,000 $km^2$, King County is home to a wide range of ecoregions and a diversity of freshwater and marine (aquatic) habitats. With more than 2.3 million human residents, King County is also among the most populous U.S. counties and has seen population growth at 20% since 2010. Recent publications reporting chemicals of emerging concern (CECs) in King County's surface waters, fish tissue and marine sediments of Puget Sound demonstrate that a growing list of unregulated anthropogenic chemicals intersect the county's valued natural resources. To date, efforts to describe CECs in King County's aquatic habitats have employed study designs typical of those used for chemicals we know well, like persistent organochlorines, metals, and polycyclic aromatic hydrocarbons. However, CECs span an enormous range of chemical properties, toxicities, and environmental fates. Sources of CECs include the obvious (pharmaceuticals in wastewater) and those hidden in plain sight (vehicle tires). To better prioritize and address CECs across the county, we developed a systematic approach to CEC study design based on a conceptual framework of CEC uses, mobility, fate and toxicity. By first considering land use, the specific CEC source types within each land use, mechanisms and pathways of transport to aquatic habitats and specific ecological receptors, more targeted study designs can be developed. Using this approach, specific conditions – including any ecological risks – will be better described and defined. The current framework is presented with examples of its application and the outlines of resulting study designs. The framework is a work in progress, and we invite your feedback. A more strategic approach to studies of CECs in aquatic habitats will generate data at the level of specificity needed to prioritize the CECs and their sources for study or management, and to address related threats to aquatic ecosystems.

**2.03.P-Mo057 Multi-Region Assessment of Chemical Mixture Exposures and Predicted Cumulative Effects in USA Wadeable Urban/Agriculture-Gradient Streams**

***Paul M Bradley****, Celeste Journey, Kristen M. Romanok and Kelly L. Smalling, U.S. Geological Survey*

Freshwater stream ecosystems in urban/agriculture-developed watersheds reflect aggregated impacts of multiple in-stream stressors derived from multiple sources. Limited data on possible adverse biological effects of contaminant mixtures in developed headwater streams are recognized challenges to management of multi-stressor impacts in freshwater fluvial networks. Chemical-contaminant mixture compositions and aggregate biological effects are less well understood in small headwater streams, which comprise most of stream length, riparian connectivity, and spatial biodiversity. During 2014-2017 U.S. Geological Survey (USGS) measured 389 unique organic analytes (pharmaceutical, pesticide, organic wastewater indicators) in 305 headwater streams within four contiguous United States (US) regions. Potential aquatic biological effects were evaluated for estimated maximum and median exposure conditions using multiple lines of evidence, including cumulative risk screening based on vertebrate-centric ToxCast TM exposure-response data and on invertebrate and nonvascular plant aquatic life benchmarks. Mixed-contaminant exposures were ubiquitous and varied. Contaminant summary metrics correlated, strong-positive (rho (r): 0.569-0.719) to multiple watershed-development metrics, only weak-positive to point-source discharges (r: 0.225-353), and moderate- to strong-negative with multiple in-stream invertebrate metrics (r: -0.373 to -0.652). Risk screening indicated common exposures with high probability of vertebrate-centric molecular effects and of acute toxicity to invertebrates, respectively. The results confirm exposures to broad and diverse contaminant mixtures and provide convincing multiple lines of evidence that chemical contaminants contribute substantially to adverse multi-stressor effects in headwater stream communities.

**2.03.P-Mo059 Prioritizing Organic Contaminants and Locations of Ecological Concern using Sediment from Lower Rio Grande Valley Resacas**

***Sarah Nash**, Seenivasan Subbiah and Jordan Crago, Texas Tech University*

Over the last half century, nearly 95% of the Lower Rio Grande wildlife habitat has been replaced by human and agriculture development, leading to increased anthropogenic contaminant loading into the remaining freshwater habitats. Although there are several studies on the Rio Grande, little is known about the impact of emerging and legacy contaminants in resacas (oxbows) along the US-Mexico border in the Lower Rio Grande Valley (LRGV), Texas, and the impacts they have on ecosystem biota. These resacas are important freshwater systems for endemic species and act as drinking water reservoirs and in some cases a source of food for the surrounding communities. The goal of this study is to identify and characterize emerging and legacy contaminant loading along select resacas along the US-Mexico border and their effects on aquatic organisms. In this study, we collected sediment samples at 13 different sites from LRGV resacas across the dry and wet season of 2021. Invertebrate, *Lumbriculus variegatus*, and vertebrate, zebrafish, toxicity testing was performed, and chemical analysis of over 100 non-point source pollutants was performed to determine changes in chemical mixture composition across time. Chemical analysis of the sediment indicated there were resaca-specific differences based on surrounding land use patterns. PAHs, PCBs, pyrethroids, and organochlorines were found in sediment at most sites during spring, summer, and fall seasons but with varying concentrations dependent on rain events. High concentrations of PAHs were measured during summer and fall, and decreased during spring sampling events. The potential toxicity of these sites was evaluated by calculation of toxicity equivalency quotients (TEQs). TEQs were calculated from sediment PAH and PCB data indicating most toxicity present at sites 9 and 10 (urban) during spring and summer and sites 2 and 12 (peri-urban) during fall. Zebrafish embryo toxicity testing was performed on water samples and sediment extract. Toxicity testing results indicated that there were site-specific and season-specific differences in behavior (startle-response) and morphological deformities. Toxicity data for sediment invertebrates and zebrafish will be presented as part of a toxicity identification evaluation (TIE) for prioritization of organic contaminants of concern.

**2.03.P-Mo060 Influence of Salinity on the Bioconcentration of Six Aquatic Pollutants**

***Scott St. Romain**[1], Sara J. Hutton[2], Emily Pedersen[2], Patrick Chappell[2], J White[2], Kevin Armbrust[1] and Susanne M Brander,[2] (1) Louisiana State University, (2) Oregon State University*

Rising sea level and changes in precipitation patterns are often linked with changes in salinity in estuarine systems. Studies have shown the bioconcentration factor (BCF) of aquatic pollutants can vary between seawater and freshwater systems. Consequently, differences in toxicity and bioconcentration of compounds across salinity gradients are a topic of increasing interest when assessing risk to estuarine fish species. Since an increase in salinity can increase the octanol–water partition coefficient (log Kow) of aquatic pollutants, which makes them less hydrophilic and more lipophilic, we hypothesize that aquatic pollutants will have a higher affinity for the lipids of an organism in higher saline vs lower saline water. Therefore, we are conducting a bioaccumulation study using model fish roe at 5 practical salinity units (PSU) and 25 PSU to six commonly used pesticides: bifenthrin, chlorpyrifos, dicloran, myclobutanil, penconazole, and triadimefon. Previous studies with these compounds demonstrate significant differences in mortality to triadimefon between 5 and 15 PSU and differences in behavioral toxicity to bifenthrin at different salinities, which suggests salinity does influence toxicity. Current exposure concentrations are set at each compound's LC25 values based on exposures from Inland Silversides (*Menidia beryllina*), a model estuarine organism, at 5 PSU which were determined in a previous study. Pesticides were extracted from the model roe using a modified QUECHERS method and subsequently analyzed using GC/MS/MS. Results will help elucidate whether salinity plays a significant role in the bioconcentration of aquatic pollutants. Here, we present our bioconcentration data on the six pesticides at these two salinities. Early results from triadimefon, myclobutanil, and dicloran support our hypothesis that bioconcentration increases with increased salinity. These data will provide knowledge to risk assessors and managers of estuarine and marine habitats where data on bioconcentration across salinity gradients are lacking.

## 2.03.V Assessing Contaminant Effects in Ecosystems with Multiple Stressors

### 2.03.V-02 Interpopulation variation in nitrite tolerance in the eastern mosquitofish (Gambusia holbrooki)

***Oriol Cano-Rocabayera*[1]**, *Kevin J Kroll*[1], *Jonas Jourdan*[2] *and Nancy Denslow*[1], *(1) University of Florida, (2) Johann Wolfgang Goethe-Universität Frankfurt am Main, Germany*

Nitrogen pollution is a major constituent of global change, threatening local biodiversity, ecosystem services, and causing serious environmental damage. Specifically, in areas with heavy agricultural soil-use, excessive use of nitrogen fertilizer pollutes the groundwaters with nitrates, but also with ammonia and nitrites. Freshwater fish and other aquatic fauna are especially vulnerable to nitrites, which can cause massive mortalities at even low concentrations < 0.1 mg/l $NO_2^-$ - N. Adaptation of fish to environments with relatively high concentrations of chemicals has occurred throughout the history of life, although contemporary evolution acts at a much more rapid pace. The growing use of land for mass agriculture and livestock industries in the last 50 years in Florida has dramatically increased the nutrient loading into the groundwaters that feed the springs. Nitrite poses a serious threat for freshwater fauna as it is rapidly up taken and disturbs ion homeostasis and blood gas transport in fish. In this study, we evaluated, by means of a common-garden experiment, the tolerance of fish to nitrite using three different populations of eastern mosquitofish (*Gambusia holbrooki*) with different background nitrogen pollution histories. Mosquitofish females were exposed to nitrite in the lab, to either < 0.005 mg/l $NO_2^-$ (control) or 0.3 mg/l $NO_2^-$ for ten days and we assessed at the end of the exposure period their blood $O_2$ transport capacity by measuring the concentration of four different types of hemoglobin, their total hematocrit, and their respiratory rates. Preliminary results show slight but significant varying patterns in the response of the exposed fish, depending on the population source, as evidenced by their respiratory rates and the blood erythrocyte counts. Mortality was very low, and hemoglobin profiles indicate high tolerance of *G. holbrooki* to nitrite contamination – a factor supporting their invasion success in agriculturally dominated regions around the world.

### 2.03.V-03 Effects of Microplastics (LPDE, PLA) on Mysid Shrimp (Americamysis bahia) in combination with temperature: A physiological perspective

***Felix Biefel*[1,2,3]**, *Susanne M Brander*[3], *Richard Connon*[2] *and Juergen Geist*[1], *(1) Technical University of Munich, Germany, (2) University of California, Davis, (3) Oregon State University*

Research and concerns about the toxicity of microplastics has increased recently, however, there is still a lack of understanding concerning effects on aquatic wildlife.

In this study, mysid shrimp (*Americamysis bahia*), a key species in estuarine and marine food chains, were exposed to 3 particle concentrations (5, 50, 500 particles/ml), the lowest being environmentally relevant, with a size of 1-20 microns. In the context of climate change, three different temperatures 22, 25, and 28 °C were investigated at a salinity of 15 ppt. Endpoints measured in response to LDPE (low density polyethylene), PLA (Polylactic acid), and possibly further particle types (nano, fibers, tire particles, cotton) included mortality, particle uptake, organismal growth, weight, and swimming behavior, as well as rates of reactive oxygen species (ROS). As part of this study, we also evaluated post-exposure swimming behavior within an oxygen deficient environment, to represent physical attributes of microplastic exposure, such as particles clogging the gills.

We hypothesize that microparticles cause negative effects on fitness-relevant endpoints, in a concentration-dependent manner, resulting in impaired swimming activities, feeding, growth, and capability in low oxygen environments. The 28 °C treatments are expected to cause more stress due to increased metabolism and therefore uptake. Initial trials, without microplastic exposure, suggest that there is greater activity at higher temperatures. The evaluation of microplastic exposure across a range of temperatures is important as it is expected that increased temperatures will pose greater challenge to aquatic wildlife also coping with pollutant exposure.

**2.03.V-04 Potential Health Effects of Contaminant Mixtures from Point and Nonpoint Sources on Fish and Frogs in the New Jersey Pinelands**

***Sara Breitmeyer***[1]*, Heather L. Walsh*[1]*, Vicki Blazer*[1]*, John Bunnell*[2]*, Patrick Burritt*[2]*, Jeff Dragon*[2]*, Michelle L. Hladik*[1]*, Paul M. Bradley*[1]*, Kristin Romanok*[1] *and Kelly L. Smalling*[1]*, (1) U.S. Geological Survey, (2) New Jersey Pinelands Commission*

Aquatic ecosystems comprise complex contaminant mixtures from anthropogenic pollution on a global scale. Point (e.g., municipal wastewater) and nonpoint sources (e.g., stormwater runoff) are both drivers of contaminantmixtures in aquatic habitats. The objectives of this study were to identify the contaminant mixtures present in surface waters impacted by both point and nonpoint sources, to determine if amphibian and fish health effects (testicular oocytes and parasites) occurred at these sites, and to understand if differences in biological and chemical measures existed between point (on-stream) and nonpoint sources (off-stream). Water chemistry samples, native and non-native fishes and native frogs were collected from 21 sites in the New Jersey Pinelands, United States. Sites were classified based on percent of surrounding altered land (upland agricultural and developed). Reference sites were surrounded by <4% and degraded sites were surrounded by 12–75% altered land. Off-stream sites consisted of 3 reference and 10 degraded wetlands. On-stream sites consisted of 2 reference lakes and 6 degraded streams/lakes (4 sites above and 2 sites below wastewater outfalls). Surface water was collected 4 times at each site and analyzed for 133 organic and inorganic contaminants. Detected organic contaminants consisted of steroids, phytochemicals, pesticides, personal care products, polycyclic aromatic hydrocarbons, mycotoxins, and industrial chemicals and inorganic contaminants included toxic heavy metals and metalloestrogens. One native and 5 non-native fish species were collected from stream/lakes and native green frogs were collected from wetlands. Limited differences in contaminant concentrations were observed in reference and degraded wetlands. However, results from the streams/lakes sites indicated that landscape alteration was the primary driver of contaminant concentrations rather than municipal wastewater inputs. Incidence of estrogenic endocrine disruption (intersex) was species dependent. Parasite prevalence was site and species dependent, prevalence of eye parasites increased with increasing cumulative concentrations of organic and inorganic contaminants. In human impacted landscapes more spatiotemporal field studies are needed to assess the association between chemical mixtures and the observed biological effects for a variety of species.

**2.03.V-05 An Approach to Define Baseline Ambient Concentrations of Per-and Poly-fluoroalkyl Substances (PFAS) in Relation to Land Uses**

***Paula Sardina****, Paul J. Leahy, Simon Sharp and Minna Saaristo, Environment Protection Authority Victoria, Australia*

One challenge for environmental regulators and risk assessors is the limited availability of PFAS ‘ambient baseline’ concentration data (i.e., typical concentrations from diffuse pollution, with no direct impact from point sources) for different environmental media and spatial scales. This data is important to compare against concentrations detected at contaminated sites and to help define cleanup goals. In this study, we 1) developed a quantitative process to classify land uses for PFAS-related studies in freshwater environments, 2) defined the ambient baseline concentration of single PFAS and PFAS mixtures associated with these land-use classes and 3) undertook an ecological risk assessment using hazard quotients (HQ). PFAS (n=33) were analysed from freshwater samples collected at 98 sites across Victoria, south-east Australia. The upstream catchment land-use proportion for each site was calculated using the Victoria Land Use Information System (VLUIS) and the Australia Land Use and Management (ALUM) Classification. Multivariate techniques were used to group sites into streamlined, well-defined classes of land use (remote, agricultural, urban and mixed). We found that PFAS detections, concentrations and mixtures increased with increasing land-use intensity: remote < agricultural = mixed < urban. The remote land-use class (>85% conservation reserves in the upper catchment) had the lowest total PFAS concentration (SPFAS) (min – max = <LOR – 0.0002 ug/L), with only PFOS (0.0002 ug/L) detected in one sample. Conversely, the urban land-use class (>50% residential, industrial and/or commercial in the upper catchment) had the highest SPFAS (0.02 – 0.76 ug/L) and PFOS (0.0007 – 0.081 ug/L) concentrations. The agricultural (>60% agricultural land use in the upper catchment) and mixed (mix of urban and agricultural) land-use classes had similar SPFAS (0.006 - 0.13 ug/L) and PFOS (<LOR – 0.048 ug/L) concentrations. Of the 16 PFAS detected in water samples, only PFOS showed an elevated risk of harm (i.e., HQ>1) to ecological values at some sites. The number of sites with HQ>1 increased with increasing land-use intensity. This study provides the first comprehensive data of PFAS in freshwater environments in Victoria. It also provides streamlined and practical categories of PFAS baseline ambient concentrations that can be used for comparison against levels measured from similar environments or when assessing risks at impacted sites.

## 2.03A Assessing Contaminant Effects in Ecosystems with Multiple Stressors

### 2.03A.T-01 Best Practices for Interpreting Bioassay Results from Environmental Samples

***Bruce Duncan***[1]*, Bradley E. Sample*[2]*, Karen Kracko*[3] *and Amanda Maxemchuk*[3]*, (1) Bruce Duncan Environmental Consulting LLC, (2) Ecological Risk, Inc., (3) SRC Inc.*

Results from site-specific bioassays are an important line of evidence for evaluating ecological risks and determining site-specific effects thresholds. However, bioassays reflect the effects of multiple stressors, including non-chemical stressors such as environmental variables (e.g., pH, sediment TOC [Total organic carbon], grain size, etc.). Because of this, it can be a challenge to reliably identify chemical-specific concentrations associated with observed toxicity to be used to help set remediation goals or cleanup levels. Effects seen in bioassays may be significant, and the goal is to tease out the portion of effects due to released hazardous materials and to understand associations among parameters associated with the specific contaminant(s) of interest. The following approach is suggested as best practices for extracting information of interest from toxicity bioassays: 1) Check the assumptions and characteristics against a well-performed bioassay; 2) Match approaches with goals of the assessment and quality of data (classifying samples as toxic or non-toxic; use of reference samples; determining and using outliers; combining and partitioning sample sets); (3) Apply analytical tools to the bioassay data and environmental variables. Some tools discussed for evaluating bioassay results include Principal Components Analysis (PCA), dose-response curve fitting, bivariate plots and analyses, and reference envelopes. Case studies will be presented to illustrate approaches and tools useful in determining chemical-specific concentrations associated with site-specific bioassay results. The case studies are used to propose a framework for developing a toolkit and decision tree to help decide which tools to use and how to sequence their use.

### 2.03A.T-02 Patterns of Risk in the Upper San Francisco Estuary due to Contaminants, Pesticides, Water Quality Parameters and other Stressors

***Wayne G. Landis***[1]*, Mikayla Bowers*[1]*, Ethan Brown*[2]*, Steven Eikenbary*[1]*, Skyler Elmstrom*[1]*, Allie Johnson*[1]*, Eric Lawrence*[1]*, April Markiewicz*[1]*, Emma Sharpe*[1] *and Erika Whitney*[1]*, (1) Western Washington University, (2) University of Notre Dame*

We have built a Bayesian network relative risk model to evaluate multiple stressors and then to apply an adaptive management process to the Upper San Francisco Estuary(the Delta) in California. A major development is the building of a dataset that incorporates unique entries for water quality variables, species counts, precipitation, contaminant concentrations and appropriate GIS data. We are now using two pathways to estimating risk. One is the use of the dataset to establish relationships between macroinvertebrate structure with water quality data and contaminant exposure. This effort is based on examining water quality variables and groups of toxicants such as metals, organophosphates, neonicotinoids, pyrethroids, glyphosate and atrazine into pathways to predict effects to Striped Bass, Chinook salmon and Long-smelt using a toxic units approach to combine toxicity. To compliment the monitoring database, we have built an extensive data repository incorporating exposure-response data, the derived curves, EC50s when exposure-response data are not available, and other measurements. We will present the importance of water quality parameters in predicting macroinvertebrate community structure in specific regions of the USFE. We have also been able to rank the importance of a series of contaminants in estimate risk to the fish endpoints. This research is supported by the

Metropolitan Water District, California Department of Pesticide Regulation, State Water Contractors and the California Delta Program.

**2.03A.T-03 What are the effects of suspended sediment on freshwater mussels?**

***Jeff A. Steevens***[1]*, Weny Zhu*[2]*, Chris Barnhart*[3]*, Henry Brown*[2]*, James Kunz*[1]*, Stephen McMurray*[4]*, Andy Roberts*[5]*, Kathleen Trauth*[2]*, Binbin Wang*[2] *and Baolin Deng*[2]*, (1) U.S. Geological Survey, (2) University of Missouri, (3) Missouri State University, (4) Missouri Department of Conservation, (5) U.S. Fish and Wildlife Service*

Freshwater mussels are regarded as the most imperiled group of animals in the United States, and around 70% of species are listed as endangered, threatened, or of special concern. Human activities that lead to habitat degradation or poor water quality may contribute to the decline of freshwater mussels. Particularly, construction activities, such as road and bridge construction, may lead to increased suspended solids and sedimentation in rivers and streams and subsequently affect habitat quality of freshwater mussels. To date, the negative effects of suspended and excess deposit sediment from construction activities on freshwater mussels, especially the sensitive juveniles, remains mostly unknown. In this study, we investigated the effects of acute exposure to suspended sediments on three species, Fatmucket (*Lampsilis siliquoidea*), Arkansas Brokenray (*Lampsilis reeveiana*), and Washboard (*Megalonaias nervosa*). These three species were selected because they represent different functional behaviors (i.e., burial behaviors) in sediment. The Fatmucket was selected as a model species because it is a common, native, and widespread species in the Midwest. Three sediment types were selected, including sediments from the native mussel habitat from the Spring River, Missouri, a high clay content soil from the Ozark Highlands region, and limestone commonly used for construction. Two age groups, ~ 1-week-old and 2-month-old juveniles were exposed to a concentration series of total suspended solids (TSS) up to ~5000 mg/L for 96 h. Although juveniles produced greater amounts of pseudofeces at higher TSS concentrations (>1000 mg/L) for all sediment types, no or very low mortality rate (≤5%) was observed even at highest TSS concentrations for both age groups. Our results revealed that short-term exposure to high TSS concentrations of the three sediments may not cause lethal impact on the juvenile mussels. Future studies will focus on understanding the chronic effects of suspended sediment and effects of burial on juvenile mussels.

**2.03A.T-04 Assessing Benthic Invertebrate Community Response to Multiple Anthropogenic Stressors in the Presence of Habitat Variability: An Adaptive Management Perspective**

***Jennifer Ings****, Patrick Schaefer and Jeff Row, Minnow Environmental Inc, Canada*

Aquatic communities are complex and structured by interactions among habitat features, including natural gradients, and anthropogenic disturbances. Benthic invertebrate communities (BIC) respond rapidly to changes in their habitat and are used worldwide to evaluate the effects of anthropogenic stressors on aquatic ecosystems. In order to effectively assess aquatic ecosystem condition, there needs to be an accurate understanding of the current state of BIC, and expectations for how BIC respond to relevant stressors. Traditionally, inferences on patterns in BIC have been done using either upstream/downstream comparisons or Reference Condition Approach (RCA) methods but both have shortcomings that hinder interpretation. The upstream/downstream comparisons may be able to identify changes in BIC, but are unable to differentiate whether those changes are due to natural habitat differences or stressors; while the RCA can better control for habitat, it is still challenging to identify which stressors are responsible for potential impairment in BIC using this approach. Direct investigations of water quality stressors on benthic invertebrates are often done in laboratory settings with organisms not common in most natural ecosystems. These studies are also limited in their application to adaptive environmental management (AEM), because they often do not incorporate multiple stressor information, or different habitat conditions, and do not use sensitive organisms found in specific management areas. This has led to ineffective and disjointed AEM, where communities are identified as impacted, but the drivers of the impacts are unknown. An alternative approach is to use predictive modeling which incorporates multiple stressors and variation in habitat to predict BIC in a spatially explicit context. Predictions could then be used to inform AEM and assess the outcomes of management actions. We present a conceptual flowchart for using predictive modeling of BIC to evaluate potential anthropogenic effects, to inform adaptive environmental management, and to identify key decision points in the adaptive management process along with potential outcomes/actions.

**2.03A.T-05 Redevelopment and Improvement of the Floating Percentile Model in the R Statistical Environment for the Calculation of Site-Specific Sediment Quality Benchmarks**

***Claire Detering**, Brian G. Church and John Toll, Windward Environmental LLC*

The Floating Percentile Model (FPM) is a tool that generates sets of sediment quality benchmarks (SQBs) using site-specific sediment chemistry and bioassay data. Originally developed in 2003 by a Pacific Northwest group called the Regional Sediment Evaluation Team (RSET), the FPM was later adopted by the Washington State Department of Ecology (Ecology) to calculate freshwater sediment management standards. However, Ecology's Microsoft Excel®-based FPM tool is not widely accessible, and the user interface does not facilitate practitioner interpretation or optimization of the output SQBs. Windward Environmental has redeveloped the FPM tool in R, fixing a software error and addressing potential statistical problems that we identified in Ecology's version, and will present this updated and accessible version. In brief, the FPM works by identifying and selecting chemicals that have higher concentrations among toxic samples and generating SQBs for those chemicals. SQBs are derived by calculating a preliminary set of benchmarks at a low percentile of the empirical chemistry data, testing how well that set of benchmarks predicts toxicity, and iteratively increasing each benchmark until acceptability criteria are no longer met. Each preliminary benchmark becomes locked into place when it reaches its highest value that allows the set of preliminary benchmarks to pass the acceptability criteria. The final SQBs are set at the closest empirically observed concentrations less than or equal to the preliminary benchmarks.

Development of the R package allowed us to thoroughly investigate how the FPM works. We paired this knowledge with the original intent of FPM SQBs—to maximize predictive accuracy when samples are toxic while minimizing false negative predictions—and developed several optimization tools to support users. These features were designed to encourage practitioners to be mindful of inputs and how adjustments can improve SQB accuracy. The FPM as developed by RSET provides better screening-level SQBs than most consensus-based benchmarks (e.g., probable effects concentrations) or other empirical benchmarks, because FPM SQBs are generated from site-specific data using reproducible methods. Open-source R code makes the FPM available to anyone and facilitates the development of screening-level SQBs that can easily be updated (or corroborated) as new data become available.

**2.03A.T-06 Multi-Tiered Assessment Discerning Anthropogenic From Natural Stressors: Improving Causality in Risk Assessments**

***G. Allen Burton Jr.**[1], Elizabeth Nichols[1], Daniel Lavoie[2], Jon Tortomasi[2] and Steven Brown[3], (1) University of Michigan, (2)Jacobs Engineering Group Inc., (3) The Dow Chemical Company*

A multi-tiered study was conducted over several years to characterize groundwater to surface water exposures of benthic invertebrates to contaminants of potential concern (COPCs) including ammonia, pH, total dissolved solids, and chloride. Exposure resulted from groundwater exfiltration of brine from an unlined settling basin to an adjacent lake and river. Hydrogeological and ecotoxicological studies were done to assess and differentiate the effects of COPC exposure from the effects of seasonal reductions in dissolved oxygen (DO) levels. Stations were sampled repeatedly along the shoreline of the lake and river adjacent to the settling basin, and at several least-disturbed reference sites. Assessment lines-of-evidence included: 1) Physicochemical characterization of near-surface porewaters and near-bottom surface waters; 2) Benthic invertebrate community indices using grab samples and reciprocal transplant methods; 3) Laboratory worst-case scenario toxicity testing; 4) *In situ* toxicity testing; 5) Diurnal monitoring of DO; and 6) Manipulation of DO in limnocorrals with concurrent *in situ* toxicity testing. Surprisingly, benthic communities at least-disturbed reference sites were also found to be impaired/degraded, suggesting biological conditions were negatively affected by stressors other than COPCs. Comparisons of water quality data to results from *in situ* toxicity tests showed that, in many cases, observed biological effects were not related to COPCs. Results from diurnal monitoring showed that shoreline habitats in the study area, including at reference sites, exhibited substantial fluctuations in DO especially during summer. Studies manipulating DO in limnocorrals with concurrent *in situ* toxicity testing showed DO was an important stressor influencing benthic populations. Observations of elevated ammonia levels in near-surface porewaters and near-bottom surface waters, and results from reciprocal transplanting of benthic invertebrates among stations, suggested that ammonia could be a factor affecting benthic communities at one or two stations immediately adjacent to the settling basin. Otherwise, the effects of COPCs could not be differentiated from the influence of naturally caused low DO. These findings highlight the importance of utilizing multiple lines-of-evidence over time to effectively assess and distinguish between the effects of anthropogenic and natural stressors.

**2.03A.T-07 Ecological consequences of neonicotinoid mixtures in U.S. streams.**
***Travis S. Schmidt***[1]*, Janet L. Miller*[2]*, Barbara Mahler*[1]*, Peter Van Metre*[1]*, Lisa Nowell*[1]*, Mark Sandstrom*[1]*, Daren Carlisle*[1]*, Patrick Moran*[1] *and Paul M. Bradley*[1]*, (1) U.S. Geological Survey, (2) Bureau of Land Management, National Operations Center*
Neonicotinoid mixtures are common in streams worldwide, but corresponding ecological responses are poorly understood. We took a team approach melding scientistic disciplines (chemistry, hydrology, ecologist, statistics) and approaches (experimental, observational, modeling) to narrow this knowledge gap. A mesocosm experiment determined that concentrations of the neonicotinoids imidacloprid and clothianidin (range of exposures 0 – 11.93 µg/L) above the Hazard Concentration for 5 percent of species (0.017 µg/L and 0.010 µg/L, respectively) caused a loss in taxa abundance and richness, disrupted adult emergence, and altered trophodynamics, while mixtures of the two neonicotinoids caused dose-dependent synergistic effects. This team surveyed 85 Coastal California streams for a broad suite of pesticides (over 250 parent compounds including 7 neonicotinoids) and invertebrate communities. Neonicotinoids were commonly detected (59% of samples (n=340), 72% of streams), frequently occurred as mixtures (56% of streams), and potential toxicity was dominated by imidacloprid (maximum = 1.920 µg/L) and clothianidin (maximum = 2.510 µg/L). Multivariate ecological response models derived from field observations were consistent with the synergistic effects observed in the mesocosm experiment, indicating that neonicotinoid mixtures pose greater than expected risks to stream health.

**2.03A.T-08 Discussion - Assessing Contaminant Effects in Ecosystems with Multiple Stressors**
***David Ostrach***[1]*, Lawrence Kapustka*[2] *and Cameron A. Irvine*[3]*, (1) Ostrach Consulting, (2) LK Consultancy, Canada, (3) RBI*

**2.03B Assessing Contaminant Effects in Ecosystems with Multiple Stressors**

**2.03B.T-01 Beyond the Roads: Assessing land use factors that influence road runoff toxicity.**
***Julann A. Spromberg****, Blake Feist and Nathaniel Scholz, National Oceanic and Atmospheric Administration (NOAA) Fisheries Northwest Fisheries Science Center*
Salmon conservation in urbanizing watersheds poses unique challenges related to habitat availability, water quantity, and water chemistry. Urban stormwater is composed of thousands of chemicals and is often directly discharged into freshwater habitats. A primary constituent is road runoff, which has been implicated in the coho salmon mortality syndrome - the pre-spawn mortality of adult coho salmon returning to their home streams. Previous analyses looked at the relationship between the mortality syndrome (time series of coho spawner survey data from 51 streams distributed across an urban gradient) and basin scale habitat conditions (time series of nationally available geospatial data) to identify the landscape characteristics associated with the syndrome. Structural equation modeling revealed an urbanization gradient associated with road density and traffic intensity, among other variables, positively related to mortality. The resulting predictive map identified mortality rates in unmonitored basins, and an expected loss of spawners in developed landscapes. Recently, the

tire-derived chemical 6-PPD-quinone has been identified as the acutely toxic causative factor in the coho mortality syndrome in urban watersheds. Thousands of sites in basins with predicted coho mortality are proposed for restoration and reconnection, particularly through removal of fish passage barriers (e.g. culverts). Restoring access to habitat with poor water chemistry may create a drain on nearby populations and result in a net loss of abundance. Coho salmon exhibit metapopulation dynamics and straying links populations inhabiting high and low quality habitat such that poor habitat patches can create a drain on populations in good habitat. We propose to identify and test hypotheses about land use factors and infrastructure on a catchment scale with refined spatial data that will help prioritize conservation sites with water chemistry unimpaired by road runoff.

**2.03B.T-02 Multigenerational toxicity of pyrethroids at two salinities in the model estuarine fish, Inland Silverside (Menidia beryllina)**

***Sara J. Hutton***[1]*, Samreen Siddiqui*[1]*, Christopher Markgraf*[1]*, Emily Pedersen*[1]*, Amelie Segarra*[2]*, Michelle L. Hladik*[3]*, Richard Connon*[2] *and Susanne M Brander*[1]*, (1) Oregon State University, (2) University of California, Davis, (3) U.S. Geological Survey*

Climate change is causing changes in precipitation patterns and increased sea levels. These alterations are linked with increased salinity in estuaries, making the potential differences in toxicity across a salinity gradient a topic of increasing interest in assessing risks to estuarine species. As ionic concentration increases, the log $K_{ow}$ and water solubility of a compound has been shown to increase and decrease, respectively. Pyrethroid insecticides are commonly used globally in agricultural, industrial, and household settings and are detected in freshwater, estuarine, and marine ecosystems. Recent studies have shown that pyrethroid toxicity changes across a salinity gradient. Early-life stage fish exposures to pyrethroids cause multigenerational toxicity at environmentally relevant concentrations. The aim of this study was to assess multigenerational toxicity of pyrethroids at salinities relevant to estuaries. We exposed Inland Silversides (*Menidia beryllina*) from 5 days post fertilization (~ 1-day pre-hatch) for 96 hours to 1 ng/L of bifenthrin, cyfluthrin, or cyhalothrin at two salinities relevant to estuaries (6 and 10 PSU). Following exposure, a subset of F0 larvae were analyzed for behavior and growth index. The remaining F0 larvae were either saved for gene expression analysis and acetylcholinesterase (AChE) inhibition or reared in clean water until they reached sexual maturity. After mature fish spawned, F1 larval samples were collected. Fecundity, sex ratio, and gonadal-somatic-index (GSI) were assessed in F0 adults. F1 larvae underwent the same behavioral, growth, and molecular assessments as the F0s. Preliminary results suggest that all pyrethroids had a significant effect on behavior and F1 development, but effects differed depending on salinity. Additionally, we found a significant reduction in GSI in the 6 PSU fish exposed to bifenthrin and cyhalothrin which was not found in the 10 PSU exposures. Salinity alone has been shown to alter gene expression, which may alter the rates of metabolism and could explain the differences in toxicity found here. Here we present our results on behavior, development, gene expression, and AChE inhibition at the two salinities. These data can be used by risk assessors interested in pyrethroid toxicity in estuaries as well as those interested in multigenerational effects.

**2.03B.T-05 Development of a national-scale model to predict environmental mercury risk using dragonfly larvae as biosentinels**

***Christopher Kotalik***[1]*, James Willacker*[1]*, Jeff Wesner*[2]*, Brenden Johnson*[1]*, Colleen Flanagan Pritz*[3]*, Sarah Nelson*[4]*, David Walters*[1] *and Collin Eagles-Smith*[1]*, (1) U.S. Geological Survey, (2) University of South Dakota, (3) National Park Service, (4) Appalachian Mountain Club*

Mercury (Hg) contamination is a risk to environmental health, but predicting Hg exposure at the landscape-scale is difficult due to high variability in abiotic and biotic factors that influence Hg methylation and bioaccumulation. The Dragonfly Mercury Project (DMP) is a citizen-science program that monitors Hg at a continental scale in U.S. National Parks and other public lands using dragonfly larvae as biosentinels, providing significant spatiotemporal coverage of Hg accumulation data across a range of environmental conditions. Using Hg concentrations collected from more than 20,000 dragonflies (>1,200 site-years) across the U.S., we are developing a landscape-scale model to predict Hg bioaccumulation (i.e., exposure risk) using a Bayesian hierarchical modeling approach. The model variables include landscape features (e.g., landcover, wetland extent, watershed soil carbon), water chemistry (e.g., DOC, pH, sulfate, nitrate), and varying effects of habitat and ecoregion that account for context-dependent responses to Hg exposure. The goals for this modeling effort include predicting Hg risk in unsampled water bodies, forecasting risk by manipulating model inputs, iteratively updating the model with future collection events, and the integration of this model into a dashboard tool. We will present on the ancillary data acquisition and harmonization, constructing and training the model, and preliminary model results.

**2.03B.T-06 Effects of Lifetime Hypoxia Exposure on Mercury Uptake Using the Elemental Composition of Fish Otoliths, Eye Lenses, and Muscle Tissues**

***Hadis Miraly****, N. Roxanna Razavi and Karin E. Limburg, SUNY College of Environmental Science and Forestry*

For decades, chemical analyses of calcified structures such as ear stones (otoliths) have elucidated the life history of fishes. More recently, other chronometric structures are increasingly being studied in this way. Among these structures, the eye lens is an interesting candidate for providing chemical information complementary to otoliths. Like otoliths, eye lenses grow throughout the life of a fish; yet unlike the aragonitic otolith, eye lenses are made entirely of protein. Further, eye lenses take up mercury (Hg) preferentially. Mercury is also a potential tracer of hypoxia as conversion to methylmercury is favored under low oxygen conditions (hypoxia), increasing Hg bioavailability to the food web. To study changes in Hg exposure through time and its potential as a tracer of hypoxia, eye lenses and otoliths from Round Goby (*Neogobius melanostomus*) of Lake Erie (n = 60), the St. Lawrence River (n = 30), and the Baltic Sea (n =30) were collected. Otoliths were used to age fish, track habitat, and assess hypoxia exposure. Eye lenses and otoliths were analyzed by laser ablation inductively coupled mass spectrometry (LA-ICP-MS); muscle tissues were analyzed for total Hg using atomic absorption spectrophotometry (AAS). The proportion of eye lens that relates to the corresponding otoliths annulus were determined, and distances were related to corresponding Hg concentrations. Using this technique, we demonstrate a method to assess Hg concentration using eye lens chronology and map Hg and hypoxia exposure history for each individual fish. We found that eye lens Hg concentrations changed as fish aged. Unlike muscle tissue that provides recent Hg exposure history, eye lenses represent a continuous record of lifetime Hg exposure and hold great promise for quantifying individual exposure to environmental stressors.

**2.03B.T-08 Multiple stressor effects on freshwater invertebrate communities: does warming enhance pharmaceutical active compound toxicity at the community level?**

***Claire Duchet***[1]*, Kateřina Grabicová*[1]*, Vojtěch Kolář*[1]*, András Csercsa*[1]*, Barbora Zdvihalova*[2]*, Tomáš Randák*[1] *and David Boukal*[1]*, (1) University of South Bohemia, Czech Republic, (4) Biology Center CAS, Czech Republic*

Predicting the effects of combined stressors on ecosystems is an urgent task given the increasing anthropogenic pressures on the Earth biota. Climate warming and chemical pollution are two widespread stressors of freshwater biota. The impact of the latter is likely to increase due widespread use of cocktails of various chemical compounds and their low removal efficiency in water plants, which is particularly relevant for many pharmaceutical active compounds (PhACs).

We assessed the combined effects of warming and a mixture of PhACs on a freshwater community in a 2*2 factorial design (i.e. with/without PhACs and with/without warming) in heated outdoor mesocosms. We combined a single-pulse cocktail of 15 PhACs from major drug categories (cardiovascular, psychoactive, antihistaminics and antibiotics), at concentrations commonly measured in surface waters in the Czech Republic, with warming at 4°C above ambient. The experimental community was constituted by common macroinvertebrate and plankton taxa from different trophic levels (e.g., predators, filter feeders and grazers, detritivores, and primary producers). We ran the experiment twice: during the winter (October/March) and during the summer (June/August). We repeatedly measured PhAC concentrations, environmental parameters (dissolved O2, conductivity, pH, turbidity, and temperature), chlorophyll-a concentration, zooplankton density and aquatic insect emergence (the latter occurred only in summer). At the end of the experiment, macroinvertebrates were destructively sampled.

Although the PhACs concentrations in the water decreased during the experiments, both warming and the PhACs altered the invertebrate community composition and insect emergence patterns in the summer experiment. The observed effects of the stressors on community structure and temporal dynamics of individual taxa were much stronger in the summer experiment and were dominated by temperature effects. Interestingly, we observed opposite effects of PhACs on insect emergence and survival patterns in heated and unheated ('control') mesocosms, and different impact of PhACs on key zooplankton groups (copepods vs cladocerans). Our results suggest that environmentally relevant PhAC concentrations can alter the impact of climate warming on freshwater biota through individual-level responses such as delayed or accelerated development and hence shifted phenologies of key taxa.

## 2.04 Canada's Oil Sands and Dilbit

### 2.04.T-02 Scope for Growth and Histopathological Alterations in Pacific Oysters (Crassostrea gigas) Exposed to Marine Diesel, Crude Oil, and Diluted Bitumen

***Anupinder Singh Hura***[1]*, Christopher J. Kennedy*[1] *and Timothy Green*[2]*, (1) Simon Fraser University, Canada, (2) Vancouver Island University, Canada*

With an expansion of petroleum transport projects planned for colder temperate marine ecosystems including the Pacific west coast of BC, the consequences of an oil spill under realistic environmental conditions need to be understood. Colder temperatures tend to lower the evaporation rate of lighter hydrocarbons, which in turn results in sessile communities being exposed to complex, toxic, and bioavailable petroleum compounds for longer durations. A representative and environmentally relevant range of exposure concentrations (0%, 25%, 50%, 100% of a water accommodated fraction) and duration (7-d exposure and 28-d recovery period) were used to determine the effects of three petroleum products (marine diesel, crude oil, and diluted bitumen) on the scope for growth (SFG) and histopathology of various tissues in Pacific oysters (*Crassostrea gigas*). For SFG determinations, algal clearance rate (CR), oyster respiration rate (RR), and absorption efficiency (AE) were measured. The histopathological analysis was performed by measuring the gonadal thickness, digestive tubule, and digestive lumen length. The results from these experiments will be utilized during the development of oil spill models and response strategies, environmental risk assessments, and monitoring plans for managing marine organisms, particularly bivalves, in the event of petroleum spills in the marine environment.

### 2.04.T-03 The little things: effects of oil spills on invertebrates and early life stages of fish, a summary of ecosystem studies conducted at the IISD-Experimental Lakes Area

***Lauren Timlick***[1]*, Lisa Peters*[1]*, Sonya Marie Michaleski*[1,2]*, Patrique Bulloch*[2]*, Jamie Dearnley*[2]*, Juan Manuel Gutierrez-Villagomez*[3]*, Valerie S. Langlois*[3]*, Jose Luis Rodriguez-Gil*[2]*, Madeline Stanley*[2]*, Gregg Tomy*[2]*, Tyler Black*[4]*, Mark L. Hanson*[2]*, Phillip Ankley*[5]*, Holly Kajpust*[6]*, McKenzie Perry*[2]*, Krista Robertson*[3] *and Vince Palace*[1]*, (1) IISD Experimental Lakes Area (IISD-ELA), Canada, (2) University of Manitoba, Canada, (3) Institut National de la Recherche Scientifique (INRS), Canada, (4) University of Guelph, Canada, (5) University of Saskatchewan, Canada, (6) TC Energy, Canada*

Since 2018, IISD-Experimental Lakes Area in northwestern Ontario, Canada has been conducting model oil spills to assess the efficacy of minimally invasive methods for remediating impacted shorelines. The companion studies, Freshwater Oil Remediation Study (FOReSt) and and FLOating Wetland Treatments to Enhance Remediation (FLOWTER) study have also examined potential impacts and trajectories of whole ecosystem recovery following model spills of diluted bitumen and conventional heavy crude oil. While evaluating the efficacy of shoreline washing and sorbent collection, engineered floating wetlands, and nutrient enhanced monitored natural recovery as remediation methods, results from both of these studies have demonstrated that invertebrates and early life stages of fish are among the most sensitive organisms to the presence of polycyclic aromatic compounds (PACs), the primary compounds in oil that drive chronic toxicity. Summaries of zooplankton community analysis, emergent insect abundance and diversity, as well as benthic invertebrate communities assessed using traditional sediment grab sampling and enumeration will be provided for in-lake studies. Supporting data from model spill studies conducted in smaller systems, and pertaining to effects on invertebrates, will also be discussed. To assess effects of residual oil on early life stages of fish, fathead minnow embryos were collected 24 hrs post-fertilization and incubated in water from shoreline enclosures treated with oil for 7 days. While survival and growth were not generally affected, rates of deformities were significantly elevated in exposed embryos compared to those from reference systems. These results indicate that zooplankton, emergent insects, benthic invertebrates and early life stages of fish are the most sensitive indicators of chronic exposure to residual oil after an oil spill clean-up and make up a crucial component of any monitoring program following an oil spill.

**2.04.T-04 Chronic toxicity and bioaccumulation of trace elements in daphnids exposed to water and sediment from an oil-sands tailings pit lake.**

***Sunny Choi***[1]*, Chad Cuss*[2]*, Chris Glover*[3]*, Greg Goss*[1] *and William Shotyk*[1]*, (1) University of Alberta, Canada, (2) Memorial University of Newfoundland, Canada, (3) Athabasca University, Canada*

Lake Miwasin is a constructed pilot-scale pit lake containing treated tailings, generated by the extraction of oil from Alberta's bituminous sands. It is expected that such lakes will help to ameliorate any toxic effects of tailings as a part of their return to the surrounding landscape. The long-term goal of this research is to understand how the hydrological and geochemical processes that occur over time in a pit lake setting will affect the bioavailability, bioaccumulation and toxicity of the trace elements in treated tailings and overlaying waters. The objective of these early studies was to determine toxicity of Lake Miwasin water and sediment to the model freshwater invertebrate species *Daphnia magna* in year 1 and 2 following lake construction, and to analyze trace metal body burdens for trace elements that may contribute to the observed effects. Acute 48 h toxicity tests were conducted with mortality as the endpoint, and chronic 21 d toxicity tests were performed using reproduction and growth as endpoints. No mortality was observed over 48 h; however, final body masses were significantly larger in daphnids exposed to Lake Miwasin water. Daphnid reproduction was also affected, with reduced total neonate production observed in both Lake Miwasin water and combined water/sediment groups relative to controls. Exposure to Lake Miwasin water and combined water/sediment increased trace metal (i.e., Mo, Ni, Al, Co) burdens in daphnids. To mimic the effects of riparian runoff into the lake that will occur as this artificial ecosystem evolves, additions of commercially available sources of dissolved organic matter (DOM) were made to exposure waters. The inclusion of DOM ameliorated the effect of Lake Miwasin water on reproduction in chronic exposures. Developing a better understanding of the evolving toxicity associated with trace elements in Lake Miwasin is important for assessing the safety of future pit lakes and water releases.

**2.04.T-05 Application of biomimetic extraction (BE) as a pragmatic analytical tool to support decision making for water and sediment quality assessments involving complex organic mixtures.**

***Aaron Redman***[1]*, Thomas Parkerton*[2]*, Abraham Smith*[1]*, Daniel Letinski*[1] *and Asfaw Bekele*[3]*, (1) ExxonMobil Biomedical Sciences, Inc., (2) EnviSci Consulting, (3) Imperial Oil, Canada*

Solid phase microextraction using polydimethylsiloxane coated fibers has become a standard tool for measuring freely dissolved chemicals in complex environments. Such analyses typically focus on measuring single contaminants of concern. These passive sampling concepts have been extended to characterize bioavailability of complex mixtures including oil contaminated test media generated using water accommodated fractions that are routinely used in lab toxicity testing as well as produced waters, oil sands process affected waters, refinery and petrochemical effluents and contaminated site sediments. When deployed under non-depletive conditions, total bioavailable extracted (BE) constituents can be quantified by desorbing the mixture in the PDMS phase into a gas chromatographic system and integrating the area under the curve using flame ionization detection.

The detector response is then translated into a molar fiber concentration using dimethyl naphthalene as an external calibration standard. A key advantage of this approach is that BE serves as a surrogate measurement for additive mixture toxic body burden and resulting aquatic toxicity. Previous work has established the technical basis for application to nonpolar organic chemicals (e.g., hydrocarbon mixtures), as well as naphthenic acids. In addition, application of the BE technique to toxicity data has established PDMS-based concentration toxicity thresholds that are similar in range and magnitude to critical target lipid body burden models based on QSAR modeling. Recent work has included round robin trials to characterize inter-lab variability of BE analyses. This method has the potential to greatly streamline effluent and sediment quality assessments that typically include complex contaminant mixtures. This presentation will review the technical basis, summarize the range of applications, and make recommendations for future applications that support decision-making.

**2.04.T-06 Ecotoxicological Effects of Fluvial Erosional Sediments on the Basal Components of the Aquatic Foodweb in Oil sands: An Integrated Experimental Approach**

***Diogo Filipe Nunes Cardoso*[1]**, *Diana Campos[1], Ana Rita R. Silva[1], Patricia Silva[1], Ana Machado[1], João Pestana[1], Andreia Rodrigues[1], Fátima C.P. Simão[1], Amadeu Soares[1], Frederick Wrona[2] and Susana Loureiro[1], (1)University of Aveiro, Portugal, (2) University of Calgary, Canada*

Physical and chemical stress on aquatic biota from natural bitumen exposure in Canadian oil sands results from natural hillslope erosion and slumping of material into the rivers, affecting riverbed habitat and water quality. This bitumen input into waters depends on high flow events, driven by snowmelt or extreme precipitation, leading to physcial/chemical stress from bitumen-containing soils entering the waters. To fully understand the ecological and cumulative effects of oil sands mining activities on the surrounding aquatic ecosystem, it is essential to evaluate the inherent effects of exposure to such natural bitumen exposure events. This study aimed to evaluate the ecotoxicological potential impact associated with erosional of riverbank bitumen deposits. A series of integrated, laboratory-based ecotoxicological bioassays were conducted using aquatic organisms with different ecological and functional traits (*Daphnia magna*, *Physa acuta*, and *Vibrio fisheri*). All these organisms were exposed to elutriates produced from natural bitumen collected from four different sources from regional rivers: fresh bitumen collected from shoreline outcrops at upper and lower reaches of the Steepbank River and the lower-Ells River and aged, fluvially processed/weathered bitumen from the shoreline of the Athabasca River. Heterogeneity of toxicity and chemical composition from bitumen collected in different regions followed the same observation in previously published field studies. Bitumen collected on the banks of the Ells River produced the most toxic elutriate, which corresponds with the higher presence of contaminants, especially naphthenic acids and more toxic PACs. Considering these results, a more detailed approach using mesocosm exposures was conducted, evaluating the effects of Ells bitumen material in sediment on natural benthic invertebrate communities. Results demonstrated that effects were mainly related to exposure to fine bitumen particles that reduced the survival of filter feeders and collectors through ingestion or adsorption to gills. The absence of effects in functional parameters was also highlighted, with no observed significant impact on primary production and leaf consumption. In summary, the tests collectively revealed that natural fluvial processes that contribute bitumen containing soils to Athabasca rivers could provide a significant input of physical and contaminant related stressors to aquatic systems, inducing adverse effects on biota.

**2.04.T-07 The effects of petroleum exposure on DNA damage and gene expression in the Pacific Oyster, Crassostrea gigas.**

***Isabella Borea*[1]**, *Christopher J. Kennedy[2], Vicki Marlatt[2] and Timothy Green[3], (1) Environment and Climate Change Canada, (2) Simon Fraser University, Canada, (3) Vancouver Island University, Canada*

The increase in petroleum use and proposed petroleum expansion projects on the Pacific West Coast of British Columbia will increase the risk of oil spills in the marine environment. Recent advances in the field of genetic toxicological tools have resulted in molecular endpoints becoming a key line of evidence in assessing sublethal effects in organisms, particularly regarding the use of genetic biomarkers. Genetic biomarkers are especially helpful for integrating the toxic effects of chemical mixtures and can therefore be applied to whole-oil exposures. In this study, Pacific Oysters were exposed to 3 different oils (marine diesel, crude, and diluted bitumen) for 7 d using water accommodated fractions (1:30 oil/water ratio) diluted at 25%, 50% and 100% with seawater. DNA damage analysis was performed immediately after termination of exposure (7d) and again at 21d and 35d post-exposure once oysters were returned to pre-exposure conditions to monitor recovery. DNA damage was quantified using the Alkaline Comet Assay by extracting oyster hemolymph from the heart cavity to assess the level of single and double stranded breaks within each treatment group. DNA damage parameters (% tail intensity, tail length, tail migration and olive tail moment) were determined for each WAF concentration group, exposure timepoint and oil product. Changes in the expression levels of stress response genes in oyster gill tissue (*ARNT, CYP1A1, CYP2C3, GST-theta-1, mGST-2, CuZnSOD, hsc70)* were also analyzed via qPCR between treatment groups. Differences in DNA damage levels were observed between exposure concentrations and time points, but there was no significant trend with increasing concentration. Gene expression levels also varied between treatment groups. This data addresses the genotoxic effects of chemicals in petroleum and the potential application of genetic biomarker data in informing fisheries management decisions.

**2.04.T-08 Non-Lethal Mucus and Caudal Fin Sampling of Fathead Minnow to Assess Oil Exposure Effects**

***Juan Manuel Gutierrez-Villagomez***[1]*, Jacob Imbery*[2]*, Tuan Anh To*[1]*, Anita Thambirajah*[2]*, Vince Palace*[3]*, Gaëlle Triffault-Bouchet*[4]*, Caren C. Helbing*[2] *and Valerie S. Langlois*[1]*, (1) Institut National de la Recherche Scientifique (INRS), Canada, (2) University of Victoria, Canada, (3) IISD Experimental Lakes Area (IISD-ELA), Canada, (4) Centre d'Expertise en Analyse Environnementale du Québec (CEAEQ), Canada*

The oil industry is economically important worldwide; however, there are still concerns regarding the risk of oil spills and debates on how to best clean and monitor the recovery of oil spills. Toxicity monitoring typically requires destructive sampling, costly tissue analysis, and delays in obtaining ecosystem recovery status data. Thus, the objective of this work is to investigate if non-lethal fish sampling, such as mucus and caudal fin punches, can be used as robust indicators of oil exposure and remediation. For this, adult fathead minnows (*Pimephales promelas*) were exposed for four days to four dilutions of water accommodated fractions (WAF) of Cold Lake Blend dilbit (6.25, 12.5, 25 and 50%) and to WAF + COREXIT EC9580A (SHORE-WAF; 0.25, 0.5, 1, and 2%) and water only control. COREXIT EC9580A is a hydrocarbon-based surface washing agent recommended by the United States Environmental Protection Agency as a shoreline cleaner after an oil spill. Total polycyclic aromatic hydrocarbons (PAH) concentrations in the WAF treatments ranged from 6-104 µg/L PAHs, while the SHORE-WAF PAHs ranged from 3-84 µg/L. At exposure completion, no mortalities were observed and liver, mucus, and caudal fin were sampled for RNA extraction and RNA-seq analyses. Preliminary results show that the WAF exposure significantly (FDR = 0.01) altered the expression of 67, 190, 292, and 3,695 genes in the liver at increasing WAF concentrations compared to the control. Similarly, the treatments affected the expression of 355, 798, 1869, and 3,629 genes in the mucus and 313, 294, 1,291 and 3,780 genes in the caudal fin compared to the control. The SHORE-WAF exposure also significantly altered gene expression, but to a lesser extent in the liver (11, 25, 74 and 116), mucus (268, 631, 306 and 579), and caudal fin (157, 866, 441 and 576) transcripts compared to the control (FDR = 0.01). Gene ontology and pathway enrichment analyses revealed several pathways that were significantly enriched (Q value = 0.05) among the different tissues including xenobiotic response, respiratory chain, and ribosomal function pathways. These preliminary results suggest that fish mucus and caudal fin punches can be used as non-lethal sampling techniques to monitor the remediation of oil-contaminated freshwater ecosystems.

**2.04.P Canada's Oil Sands and Dilbit**

**2.04.P-Th041 Plant-Enhanced Degradation of Phenanthrene: Microcosm Experiment at the IISD-Experimental Lakes Area, Canada**

***Madeline Stanley***[1]*, Aidan Guttormson*[1]*, Lisa Peters*[2]*, Blake T. Cooney*[1]*, Richard Grosshans*[3]*, Gregg Tomy*[1]*, David B. Levin*[1] *and Vince Palace*[2]*, (1) University of Manitoba, Canada, (2) IISD Experimental Lakes Area*

*(IISD-ELA), Canada, (3) International Institute for Sustainable Development, Canada*
Non-invasive methods for secondary recovery of oil spills in freshwater shorelines have been explored by the International Institute for Sustainable Development Experimental Lakes Area (IISD-ELA) and collaborators since 2018, instigated by needs to improve recovery methods. Engineered floating wetlands (EFW) were explored as a biological remediation method to enhance surface area for microbial colonization in the water column and thus enhance biodegradation of polycyclic aromatic compounds (PACs). To further investigate specific degradation activities, a microcosm experiment was conducted to determine plant-enhanced degradation of the model PAC, phenanthrene. Phenanthrene ($C_{14}H_{10}$), a three ring PAC, is a priority pollutant listed by the United States Environmental Protection Agency and is often used to detect oil contamination in the environment. Emergent plants (*Typha* sp., and two *Carex* spp.) were collected from a wetland at the IISD-Experimental Lakes Area and transplanted into 4 L jars of lake water in triplicate. After one month of establishment, experimental jars received phenanthrene standard at an initial concentration of 1 mg/L. Over six weeks of exposure, the microcosm waters were monitored for parent phenanthrene and metabolites using small volume extractions and gas chromatography-mass spectrometry. Water was also monitored for basic water chemistry and chemical oxygen demand. Plants were monitored for growth and development and the root biofilm was assessed for microbial metabolic metrics, including adenosine triphosphate and respirometry. We will compare rates of parent phenanthrene degradation between treatments to determine the effect that plant species have on biodegradation. Results from this experiment will support ongoing research at the IISD-ELA to aid in optimizing design of EFWs to best enhance degradation of PACs.

**2.04.P-Th043 Does Enhanced Monitored Natural Recovery Reduce Aqueous PACs After Primary Oil Spill Cleanup: Results from the Freshwater Oil Spill Remediation Study (FOReSt) at the IISD-Experimental Lakes Area**

***Vince Palace*[1]*, Lisa Peters*[1]*, Lauren Timlick*[1]*, Jose Luis Rodriguez-Gil*[2]*, Madeline Stanley*[2] *and Gregg Tomy*[2]*, (1) IISD Experimental Lakes Area (IISD-ELA), Canada, (2) University of Manitoba, Canada*
Traditional methods for physically removing spilled oil from freshwater shoreline environments can be ecologically damaging and delay recovery of impacted aquatic systems. However, there is uncertainty regarding the effectiveness of minimally invasive methods based on natural degradation of oil by resident bacterial communities. A Royal Society of Canada report identified the need to augment knowledge regarding emergent technologies for remediating oil spills. In response, the International Institute for Sustainable Development's Experimental Lakes Area (IISD-ELA) initiated the Freshwater Oil Spill Remediation Study (FOReSt). The FOReSt project is evaluating minimally invasive methods for cleaning oil spills from affected freshwater shorelines. Using controlled spills contained inside 5X15m enclosures in a pristine lake, we have examined methods for removing oil from freshwater shorelines by stimulating resident bacterial using nutrient addition, a method called enhanced monitored natural recovery (eMNR). Two types of crude oil were evaluated in separate field seasons: in 2019 we conducted model spills using diluted bitumen and in 2021 conventional heavy crude (CHV) was used. Recovery trajectories for concentrations of polycyclic aromatic compounds (PACs), the most toxicologically relevant components of oil, were conducted for >200d after each model oil spill. The effect of secondary remediation using eMNR on concentrations of PACs in water, sediment and biota were evaluated in 2019 following diluted bitumen model spills and in 2021 after CHV exposures. The FOReSt project will provide guidance to oil spill responders and decision makers regarding the effectiveness of minimally invasive methods for treating oil spills.

**2.04.P-Th045 Biodegradation of Polycyclic Aromatic Compounds by Engineered Floating Wetlands: Optimizing Plant Species Ratios and Nutrient Additions for Oil Spill Bioremediation**

***Aidan Guttormson*[1]*, Madeline Stanley*[1]*, Adam Scott*[1]*, Lisa Peters*[1]*, Charles Greer*[2]*, Richard Grosshans*[3]*, David B. Levin*[1] *and Vince Palace*[4]*, (1) University of Manitoba, Canada, (2) National Research Council of Canada, (3) International Institute for Sustainable Development, Canada, (4) IISD Experimental Lakes Area (IISD-ELA), Canada*

Conventional oil spill cleanup methods used for freshwater shoreline and near-shore environments can further damage the affected area and leave residual oil in the system. Consequently, less-invasive oil spill cleanup strategies are needed. Research conducted in 2021 at the International Institute for Sustainable Development - Experimental Lakes Area, Ontario, Canada, investigated the use of engineered floating wetlands (EFWs) as a minimally invasive secondary method for remediating oil spills. EFWs are floating platforms which are populated with emergent plants – the root system beneath the platform provides beneficial conditions for microbial biofilm formation and can enhance biodegradation of contaminants. Experimental EFWs were exposed to simulated oil spills in 1600 L mesocosm tubs to determine the capacity of these systems to remove oil-derived polycyclic aromatic compounds (PACs) from water and to identify design parameters which maximize EFW-enhanced oil degradation. Two EFW design parameters were studied: 1) plant species composition (*Typha* sp., *Carex atherodes*, and *C. lasiocarpa*); and 2) the carbon to phosphorus (C:P) ratio resulting from additions of fertilizer alongside an EFW. Twenty-six experimental mesocosms and EFWs were treated with 16 L of water accommodated fraction (WAF), produced by weathering conventional heavy crude oil on water for 36 hours. The systems were monitored for 13 weeks and water samples were collected to analyze PAC chemistry, including 16 PACs from the US Environmental Protection Agency priority compounds list and 28 alkylated PACs. Root samples were analyzed for microbial activity in the biofilm via adenosine triphosphate (ATP) assay and microbial community diversity via 16S and 18S rRNA gene amplicon sequencing. Initial mean concentrations of total PACs began at $176 \pm 33$ ng/L one day after WAF application and declined to baseline levels in all mesocosms by day 35. Preliminary ATP assay results indicate that biofilm activity was not affected by the presence of oil after 33 days of exposure. Ongoing analyses will identify the effects of different EFW designs and oil addition on microbial diversity and PAC removal rate. Results of this research will be communicated to industry project partners to support the use of EFWs as an alternative or supplement to conventional oil spill cleanup techniques.

**2.04.P-Th046 Quantitative source apportionment of polycyclic aromatic compounds in the Athabasca River watershed**

***Md Samrat Alam*[1]*, Jason M.E. Ahad*[1] *and Colin Cooke*[2]*, (1) Natural Resources Canada, Canada, (2) Alberta Environment and Parks, Canada*

The emissions of contaminants such as polycyclic aromatic compounds (PACs) from surface mining and upgrading activities in Canada's Athabasca oil sands region (AOSR) has raised concerns about their impact on the surrounding environment. Effective environmental management requires techniques that can accurately discriminate between different sources of PACs. Previous work demonstrated the potential of natural abundance radiocarbon ($\Delta^{14}C$) and dual ($\delta^{13}C$, $\delta^{2}H$) compound-specific isotope analysis (CSIA) to discriminate the sources of polycyclic aromatic hydrocarbons (PAHs) in particulate-bound PACs deposited in AOSR lake snowpack. Here, we build upon this work to identify and quantify the sources of PACs in surface sediments along the main stem of the Athabasca River at sites upstream, nearby and downstream of bitumen surface mining operations. Alkylated PAHs were elevated compared to unsubstituted parent PAHs, demonstrating the importance of oil sands-derived inputs from both natural and mining-related sources. Compound-specific carbon and hydrogen isotope analysis ($\delta^{13}C$, $\delta^{2}H$) will be used to assess the importance of petroleum coke (petcoke) – the carbonaceous by-product of bitumen upgrading – in riverine sediments. An additional isotope, radiocarbon ($^{14}C$), will allow for a quantitative estimate of the proportion of wildfire-derived PACs. The combined use of three isotopes ($\delta^{13}C$, $\delta^{2}H$, $\Delta^{14}C$) is expected to provide unparalleled insight into the sources of PACs in the AOSR.

**2.04.P-Th047 Comparing Polycyclic Aromatic Compounds in Air at Surface-Mining and In-Situ Mining Areas in the Alberta Oil Sands Region**

*Jasmin Schuster, **Tom Harner,** and Ky Su, Environment and Climate Change Canada*

The Athabasca Oil Sands Region in Northern Alberta is the largest deposit of bitumen in the world. The activity of the petroleum industry in the area encompasses open pit mining, in-situ mining and upgrader facilities as well as waste tailing ponds. Polycyclic aromatic compounds (PACs) are chemicals of concern released from the oil sand industrial activity. They are detrimental to human and ecological health and as semi-volatile pollutants can undergo long-range atmospheric transport. PACs such as polycyclic aromatic hydrocarbons (PAHs) have a wide range of sources in addition to the petroleum industry (e.g., wildfires); whereas, PACs such as alkylated PAHs (alkPAHs) and dibenzothiophenes (DBTs) are predominantly present in bitumen. Elevated PAC levels in the Athabasca Oil Sands Region have previously been associated with petcoke in fugitive dust from activities like open pit mining and heavy vehicle traffic. In-situ mining is becoming more predominant in in the Canadian Oils Sands Region with 80% of the deposits located too deep for open pit mining. The environmental impacts between open pit mining and in-situ mining differ.

PAC levels in air were monitored previously from 2011-2015 with both passive (19 sites) and active (3 sites) air samplers to establish a baseline in the Athabasca Oil Sands Region. Concentrations in air were in the ranges of 0.3–43, 0.15–460, and 0.04–130 ng/m3 for ∑PAHs, ∑alkPAHs, and ∑DBTs, respectively. Increasing PAC concentration in air were noted at sampling sites with increasing open pit mining activity during the sampling period. Passive air sampling was resumed in 2020 with additional sampling sites in the Peace River and Cold Lake Oil Sands Regions, where in-situ mining is the dominant bitumen extraction process. Passive air samples (polyurethane foam disks) were deployed for consecutive two months periods for March/April 2020, May/June 2020 and November/December 2021 at 13 locations – with 6 legacy sites in the Athabasca Region, 6 new sites in the Cold Lake Region and 1 new site in the Peace River Region. Location types are differentiated by impact area as background, community, open pit mining and in-situ sites. This allows the comparison of PAC patterns in air at sampling sites that are predominantly impacted by open pit mining and in-situ mining.

**2.04.P-Th048 Chronic Toxicity of Oil Sands Process Water in Early Life Stage Wood Frogs (Lithobates sylvaticus)**

*Katelyn Stenner[1], Blake Danis[1], Asfaw Bekele[2], Frank Gobas[1], Vicki Marlatt[1], Neal Tanna[3], Bonnie Drozdowski[3] and* ***Barry C. Kelly****[1], (1) Simon Fraser University, Canada, (2 )Imperial Oil, Canada, (3) InnoTech Alberta, Canada*

Oil sands process-affected water (OSPW) is typically comprised of a complex mixture of organic and inorganic constituents generated during the bitumen extraction process used by the oil sands mining industry. Naphthenic acids (NAs) are a diverse class of ionizable organic substances, thought to be the primary driver of OSPW toxicity. Recent studies have demonstrated that a biomimetic extraction via solid-phase microextraction (BE-SPME) technique may be useful for hazard assessment of OSPW, as this approach can effectively provide a surrogate measure of toxicity related to bioavailable dissolved organic contaminants. The BE-SPME method involves a relatively simple cost-effective measurement involving analysis of SPME fibers via GC-FID. The majority of OSPW toxicity studies have involved short-term acute toxicity tests in aquatic invertebrates and fish. Currently, there is a paucity of toxicity studies with amphibians. In the present study, we conducted a series of 45 d chronic early life stage wood frog toxicity experiments with OSPW/diluted OSPW (100%, 46%, 22%, 10%, 4.6%, 2%, Controls). Exposure experiments followed the protocols outlined in the OECD Larval and Amphibian Growth and Development Assay (OECD, 2015). We utilized a density of 10 tadpoles/6 L of test volume, 16:8-h light/dark cycle and feeding recently hatched tadpoles frozen kale (previously boiled) and Ward's Xenopus tadpole food ad libitum after each water change and one to three algae wafers (Hikari KYORIN Co. Inc.) once a week. Water renewals (80%) were performed every 48 to 72 hours and water quality (pH, dissolved oxygen, conductivity, and temperature) and tadpole survival were monitored daily. BE-SPME (via GC-FID) and Naphthenic Acids (via high resolution Orbitrap mass spectrometry) were measured in exposure media to assess concentration-response profiles and derive chronic effect levels (e.g., LC50, EC50). In addition to mortality, we monitored non-lethal endpoints related to growth, development and reproductive maturation. The study results provide important information regarding chronic toxicity of OSPW in amphibians. The study further demonstrates the utility of biomimetic SPME-based passive samplers for a rapid screening tool for assessing aquatic toxicity of organics in OSPW.

**2.04.P-Th049 Chronic Toxicity of Oil Sands Process Water in Early Life Stage Rainbow Trout**

*Valeria Vega[1], Katelyn Stenner[1], Asfaw Bekele[2], Neal Tanna[3], Bonnie Drozdowski[3], Vicki Marlatt[1], Frank Gobas[1] and* ***Barry C. Kelly****[1], (1) Simon Fraser University, Canada, (2) Imperial Oil, Canada, (3) InnoTech Alberta, Canada*

Oil sands process-affected water (OSPW) is typically comprised of a complex mixture of organic and inorganic constituents generated during the bitumen extraction process used by the oil sands mining industry. Naphthenic acids (NAs) are a diverse class of ionizable organic substances, thought to be the primary driver of OSPW toxicity. Recent studies have demonstrated that a biomimetic extraction via solid-phase microextraction (BE-SPME) technique may be useful for hazard assessment of OSPW, as this approach can effectively provide a surrogate measure of toxicity related to bioavailable dissolved organic contaminants. The BE-SPME method involves a relatively simple cost-effective measurement involving analysis of SPME fibers via GC-FID. The majority of OSPW toxicity studies have involved short-term acute toxicity tests. Assessments of chronic toxicity of OSPW are limited. In the present study, we conducted a series of 30 d chronic early life stage rainbow trout toxicity study with OSPW/diluted OSPW (100%, 46%, 22%, 10%, 4.6%, 2%, Controls). We also conducted additional early life stage rainbow trout exposure experiments using treated OSPW (via constructed wetland) and Athabasca river water, which served as a natural/receiving water control. BE-SPME (via GC-FID) and Naphthenic Acids (via high resolution Orbitrap mass spectrometry) were measured in exposure media to assess concentration-response profiles and derive chronic effect levels (e.g., LC50, EC50). In addition to mortality, we monitored non-lethal endpoints, including hatching success, growth (length, weight) and deformities (skeletal, craniofacial, finfold, edemas). The observed BE-SPME based concentration associated with 50% mortality in exposed rainbow trout was 22.9 mM dimethylnaphthalene/L PDMS. This was equal to 2,290 ug/L total NA's using Orbitrab. The lowest observable effect concentration (LOEC) related growth effects and deformities ranged between approximately 10 to 20 mM for BE-SPME and 1,000 to 2,000 ug/L total NA's. The observed concentration-response relationship for rainbow trout chronic OSPW exposure is comparable to those previously reported for shorter-term/acute assays using different species. The study provides important information regarding chronic toxicity of OSPW and demonstrates the utility of biomimetic SPME-based passive samplers for a rapid screening tool for assessing aquatic toxicity of organics in OSPW.

**2.04.P-Th050 Spatiotemporal Distributions of Naphthenic Acids in Surface Water From Wetlands in the Athabasca Oil Sands, Canada**

***Ian James Vander Meulen****[1,2], Danna M. Schock[3], Lukas J Mundy[1], Fardausi Akhter[1,4], Kristin M. Eccles[5], Catherine Soos[1], Kerry M. Peru[1], Dena W. McMartin[6], John V. Headley[1] and Bruce Pauli[1], (1) Environment and Climate Change Canada, (2) University of Saskatchewan, Canada, (3) Palustris Environmental, Canada, (4) Agriculture and Agri-Food Canada (AAFC), Canada, (5) University of Toronto Mississauga, Canada, (6) University of Lethbridge, Canada*

The Athabasca oil sands region (AOSR) of Alberta, Canada, is known for its unconventional petroleum production operations, as well as associated environmental management challenges. Previous studies have demonstrated that oil sands extraction activities can distribute or influence the occurrence, behaviour, and fate of environmental contaminants at a regional scale. The environmental and ecological dynamics of some regional contaminants of concern, including naphthenic acids (NAs), are poorly understood. Therefore, monitoring activities are being undertaken to examine the occurrence, characteristics, and dynamics of NAs in the region as a component of the Canada-Alberta Oil Sands Monitoring Program. This work reports the results of a 7-year study investigating NAs in the surface water of AOSR wetlands, as detected and quantified by derivatized liquid chromatography-tandem mass spectrometry. Among the wetlands sampled, recurring patterns of relative NA concentrations were detected among wetlands plausibly affected by bitumen-derived inputs, though our data did not allow us to distinguish between natural and anthropogenically-influenced NA inputs to the wetlands studied. Wetlands that formed opportunistically on or near reclamation projects (i.e., Gateway and Crane Lake) included in this study had the highest overall NA concentrations (up to 200 µg/L) as well as NA concentration distributions suggestive of bitumen-derived NA inputs. Patterns of NA concentrations consistent with those observed at Gateway and Crane Lake were also observed in undeveloped natural wetlands located above proven oil sands deposits, suggesting wetland-oil sand deposit interactions. Intra-annual sampling at individual wetlands demonstrated that NA concentrations and characteristics were inconsistent across the region, changing according to unidentified local factors. Inter-annual comparisons of individual wetlands reinforced the spatial and temporal independence of the occurrence of NAs. Although this study identifies plausible bitumen-derived NA inputs in natural wetlands in the region, the site selection here was limited and was not able to determine nor confirm the extent of bitumen-derived NA inputs into natural wetlands across the region.

## 2.04.V Canada's Oil Sands and Dilbit

### 2.04.V-01 Toxicity assessment of bottom substrate from a pilot-scale pit lake consisting of polymer-treated oil sands tailings.

***Immanuela Oluchi Ezugba**, Lorne Doig, Banamali Panigrahi, Catherine Arenas Davila and Karsten Liber, University of Saskatchewan, Canada*

The oil sand mining industry in Alberta, Canada, is currently faced with the challenge of long-term management of process by-products, including tailings and oil sands process affected water (OSPW). One promising option is incorporating these materials into pit lakes. Lake Miwasin, Suncor Energy's prototypic oil sands pit lake, was constructed using tailings (treated with a flocculant and coagulant) as the bottom substrate and capped with a blend of OSPW and fresh surface water. This study aims to assess the toxicity of the bottom substrate of Lake Miwasin to benthic macroinvertebrates. Water and sediment samples were collected from the nearshore zone and the limnetic zone of the lake at water depths of 0.3 m and 4m, respectively. ICP-MS analysis of the sediment samples in May 2020 showed that metal concentrations in both zones of the lake did not exceed the Canadian Council of Ministers of the Environment (CCME) Sediment Quality Guideline for the Protection of Aquatic Life- Freshwater.

Toxicity testing was conducted using larvae of *Chironomus dilutus* that were acclimated to reconstituted saline water (RSW) that was formulated to simulate Lake Miwasin bottom water. The animals were exposed in 14-day static-renewal sediment toxicity tests to nearshore and limnetic sediments in combination with either RSW or Miwasin bottom water. Results showed toxicity for both the limnetic (£ 5% survival) and nearshore (£ 15% survival) sediments. A whole-sediment toxicity identification evaluation (TIE) test was carried out to determine the class of toxicant(s) likely causing the observed toxicity. The results of this test were inconclusive, with no single class of contaminant identified as the primary cause of toxicity. Currently, another set of TIE tests is being conducted to help identify the potential cause(s) of toxicity associated with sediment pore water.

### 2.04.V-02 Impact of Diluent and Water Chemistry on the Methane Emission from Oil Sands Tailings Ponds

***Xiaomeng Wang**[1], Jordan Elias[1], Ian James Vander Meulen[2] and John V. Headley[2], (1) Natural Resources Canada, (2) Environment and Climate Change Canada*

The Alberta oil sands represent the third largest reserve of crude oil in the world. One method of producing this oil is by mining, which produces tailings ponds holding water, fine minerals, bitumen and residual solvents. Naturally existing bacteria consume organic compounds in the tailings ponds resulting in the emission of greenhouse gas (GHG) such as CO2 and CH4. It is estimated that the GHG emissions from tailings ponds represent 5% of the overall oil sands mine emissions. However, CH4 emissions from tailings ponds accounted for 45% of the total CH4 emissions from oil sands facilities. Given that methane is about 25 to 30 times more potent as a greenhouse gas than CO2, reducing CH4 emission from tailings ponds would have a rapid and significant effect on atmospheric warming potential from a mining operation.

Many factors may contribute to the CH4 emissions from the ponds, such as the amount and type of residual solvent (solvent is used in the bitumen extraction process) in the pond, the location of the sampling, pond water chemistry and microbial communities. In this study, we investigated the impact of diluent in the tailings materials on the methane emission and characterized the changes of microbial profile and the water chemistry. The results show a link in the reduction of sulphate and sulphur species in water chemistry with changes to the profile of naphthenic acid fraction compounds (NAFCs) in the water phase post-methane production. This observation indicates that the sulphate reduction mechanism may impact the methanogenesis process. These findings on how molecular level changes in NAFCs distribution are linked to methanogenesis, will inform future study on selection of candidate chemical amendments to help inhibit methane production in the tailings ponds.

## 2.06.P Detection, Toxicity and Environmental Risk of UV filters in Aquatic Ecosystems

### 2.06.P-Mo061 Sunscreen Chemical Concentrations in Marine Waters of the Virgin Islands National Park Pre- and Post-Ban

*__Tim Bargar__ and David A. Alvarez, U.S. Geological Survey*

Numerous studies have reported that some active ingredients in sunscreen products are toxic to corals. Because of such reports, the use of sunscreen products with those ingredients has been banned in many locations. The assumption for such bans is contamination in marine waters would be significantly reduced by the ban and the corals would be protected. The U.S. Virgin Islands is one of those locations that, beginning in April of 2020, banned sunscreen products containing the active ingredients oxybenzone, octocrylene, or octinoxate. This poster will present data from a study investigating the effectiveness of that ban. Water samples were collected from several bays (Cinnamon, Trunk, Hawksnest Bays) of the Virgin Islands National Park in March and June of 2020, December of 2021, and March of 2022. Data from those samples will be compared to sunscreen data from our prior studies in the same bays prior to the ban (2013 – 2015). Water samples from the prior studies were not analyzed for octocrylene, but they were analyzed for oxybenzone and octinoxate. Oxybenzone concentrations ranged from below detection (5 ng/L) to 6,323 ng/L and varied considerably among the sampled bays (Trunk>Hawksnest>Cinnamon) and the sampling months (June>April~December). Octinoxate concentrations, on the other hand, were much lower ranging from below detection (5 ng/L) to 140 ng/L and were less variable among the sampled bays and months. These concentrations and the apparent relations among bays and months will be compared to concentrations found after the sunscreen chemical ban.

### 2.06.P-Mo062 Can short-term data accurately represent long-term environmental exposures? Investigating multigenerational adaptation of Daphnia magna to organic sunscreen ultraviolet filters

*__Aaron Boyd__, Jessica Choi, Grace Ren, Zuo Tong How, Mohamed Gamal El-Din, Keith B. Tierney and Tamzin A. Blewett, University of Alberta, Canada*

Organic ultraviolet filters (UVFs) are an emerging contaminant of concern found in common personal care products and sunscreens to protect against harmful ultraviolet radiation. Among the most commonly used UVFs are avobenzone, octocrylene and oxybenzone. Their use in sunscreens leads to widespread environmental contamination due to leaching of these chemicals during recreational activities, posing a threat to aquatic systems with concentrations commonly reported in the range of 0.1-10 μg/L. Current research regarding the toxicity of UVFs has either used short-term exposures or high concentrations that are unlikely to be encountered in the environment. This study sought to model the long-term effects of chronic UVF exposure to the freshwater invertebrate, *Daphnia magna*, as well as their potential for acclimation through either adaptation or natural selection over four subsequent, continuously exposed generations. Initial generations of *Daphnia* showed evidence of increased physiological impairment through both a 30% decreased reproductive output and a 10-fold greater proportion of non-viable offspring. In addition, 50% mortality was observed in the first two generations of oxybenzone exposures; however, surviving daphnids proved capable of adapting to long-term UVF exposure, ultimately returning to a similar physiological state as observed in control treatments by the F3 and F4 generations. Despite these notable differences in survivourship and offspring quality across generations, minimal differences were observed between adult daphnids that survived through to the end of the F0 generation and those that did not, indicating that the eventual recovery of *Daphnia* populations to the control physiological state is a result of adaptation, rather than the weakest individuals being removed from the population via natural selection. This data suggests that *Daphnia* are capable of adapting to sublethal levels of UVFs, suggesting that perhaps current estimates of toxicity through short-term exposures are an overestimation of long-term toxicity in an environmental setting.

**2.06.P-Mo063 Realistic Sunscreen Exposure and the Consumer Usage Data Gap: A Novel Online Survey Approach Designed to Measure Consumer Application Thickness**

***Andrea Carrao*** *and James C. Coleman II, Kao USA*

In recent years, there have been numerous scientific and media publications investigating the potential impact of sunscreens and ultraviolet (UV) filters on environmental health. The potential effects of UV filters in the aquatic environment have been well studied, but environmental exposure(s) remain(s) unclear and understudied. Consumer habits and practices directly influence the amount of sunscreen products, and subsequently UV filters, potentially released into the environment. Therefore, it is critical to understand consumer usage and preferences for sunscreen products. The United States Food Drug and Administration's (FDA's) sunscreen testing methods mandate a dermal application dose for sun protection factor (SPF) determination of 2.0 $mg/cm^2$. This value is often used as a default assumption in environmental exposure assessments. However, research over the years indicates the amount of sunscreen products typically applied by consumers may be less (0.2-1.27 $mg/cm^2$). Much of the past research determined the application dose by measuring how much of the product was applied by volunteers and the applied surface area. These studies were conducted in specific sub-populations (e.g., skin cancer survivors, tourists, etc.). As such, additional research is needed to determine if these values are representative of the general population. The objective of this research was to develop a questionnaire protocol designed to produce semi-quantitative estimates of sunscreen usage by consumers in the United States and conduct a large-scale survey to determine a more realistic application dosage value for sunscreen products. Using an online platform, over 9,000 consumers were asked about their sunscreen use in general and the amount applied based on a visual reference with known dispensed sunscreen amounts. The sunscreen application dose was estimated using this visual reference (amount applied) and the volunteers' disclosed height and weight (application surface area). The desired outcome is to generate a more accurate estimate of dermal application rate of sunscreen products based on current consumer use patterns and preferences that can be used to more realistically estimate environmental exposure to UV filters.

**2.06.P-Mo064 Release and Transformation of ZnO NPs used in Surface Coatings from Pressure Treated Lumber**

***Justin Clar***[1]*, Sydney Thorton*[1]*, Sarah Boggins*[1] *and Todd Luxton*[3]*, (1) Elon University, (2) U.S. Environmental Protection Agency*

As the market for "nano-enabled" products (NEPs) continues to expand in commercial and industrial applications, there is a critical need to understand conditions that promote release of nanomaterials and their degradation materials from NEPs. Moreover, these studies must aim to quantify both the abundance and form (aggregates, ions, hybrids, etc.) of material released from NEPs to produce reasonable estimates of human and environmental exposure. In this work ZnO (NPs), commons additives in NEP surface coatings to enhanced UV protection, were dispersed in Milli-Q water and a commercially available wood stain before application to pristine and weathered (outdoor 1 year) micronized copper azole pressure treated lumber (MCA). After drying, subsamples of coated lumber were placed in synthetic precipitation solution (SPLP) for 72 hours to simulate environmental release. Trace metal analysis using ICP-OES found aged lumber released significantly less Zn than pristine lumber when using the same coating formulation. Zinc speciation analysis also demonstrates that transformation of crystalline ZnO to Zn-organic complexes shortly after application to aged lumber, and further degradation after exposure to SPLP solution. Regardless of experimental treatment, most applied zinc (>75%)

remains on the MCA surface. These experiments demonstrate the rapid transformation of NP additives once exposed to environmental systems. It is critical for studies the environmental and human health of NPs to consider the systems in which they will be applied instead of studying the pristine materials themselves.

**2.06.P-Mo065 Sensitive and robust quantification of 15 common UV sunscreen filters**

*Simon Roberts, Sujata Rajan, Jessica Smith, Karl Oetjen, Sashank Pillai, Jianru Stahl-Zeng and **Craig M. Butt**, SCIEX*

Recent research has highlighted concerns related to the potential endocrine disrupting properties of some UV filters in sun care products. In light of recent developments, the US state Hawaii is set to ban any sunscreen products that contain both octocrylene and avobenzone by January 1, 2023. In addition to this, other states in the US have already banned both oxybenzone and octinoxate because of damaging effects on the coral reefs. These developments have raised safety concerns. Therefore, it is paramount to assess the measurement limits of a range of common UV filters that are found in commercial sunscreen products. Here a rapid and robust method has been developed for the sensitive detection of 15 common UV filters.

A stock solution of 1000 µg/mL of 15 UV filter standards was prepared in methanol. A dilution series was prepared from 1 to 200 ng/mL to construct a calibration curve. Sample sunscreens (10 mg) were added to methanol (10 mL) and vortexed for 30 seconds. The resulting mixtures were centrifuged for 5 minutes (4500 RPM). The supernatant was then filtered through a 0.22 µm PTFE syringe filter. Samples were pre-spiked at 50 ppb, and post-spike sample preparation was also performed. Chromatographic separation was performed using Phenomenex Luna Omega Polar C18 analytical column (C18 100Å, 3 µm, 100 x 4.6). The MS system operated using time-scheduled multiple reaction monitoring mode (sMRM) on a triple quadrupole mass spectrometer using electrospray ionization (ESI) in positive ion mode.

This analytical method provides simple sample preparation and a reproducible method for sensitive detection of common UV filters found in commercial sunscreen products. Calibration curves were generated for 15 UV filters across a concentration range of 1-200 ng/mL. Accurate quantification was achieved across a linear range, where $r^2>0.99$, highlighting accuracy in quantification across this range. Precision and accuracy values for compounds of interest were within an acceptable criterion, with %CV < 10% and accuracy within ±30% of the expected value. LLOQ and LLOD values were as low as 5 ng/mL and 1 ng/mL in sunscreen matrix, respectively, far exceeding minimum requirements. Chromatographic evaluation shows good peak-peak separation with a total run time of 12 minutes. With regulation changes in both the US and EU, we provide an economical, robust, and reproducible method for quantification of UV sunscreen filters at a reduced measurement cost per sample.

**2.06.P-Mo066 Spatial and temporal investigation of concentrations of organic UV filters in seawater from the Florida Keys, USA.**

***Carys Louise Mitchelmore**, Andrew Heyes and Michael Gonsior, University of Maryland Center for Environmental Science*

Very few comprehensive monitoring studies have quantified levels of organic UV filters near reefs and many data gaps exist regarding spatial and temporal relationships that are critical in understanding their fate and persistence and ultimately exposure and environmental risk to resident organisms. Currently there is limited or no information available on the environmental concentrations of these chemicals in seawater from locations near coral reefs in Florida. Previous studies from other locations have found decreasing concentrations with water column depth, distance from the shoreline and in times/areas of lower recreational activity. Researchers have hypothesized that concentrations are higher in the microlayer yet there is limited or no data to support this. To address these knowledge gaps seawater samples from multiple sites (i.e. beach and reef locations) in the Florida Keys, USA were analyzed for 13 organic UV filters using LC-ESI-MS/MS for dissolved and particulate concentrations. At recreational beach locations multiple sites (and replicate samples) were assessed for concentrations in the microlayer and surface seawater at two distances from the shoreline. At one beach location a temporal assessment was conducted with samples collected early in the morning, mid-afternoon and late in the evening. At reef locations both surface and at coral depth seawater samples were analyzed. Numerous UV filters were detected in seawater with the highest concentrations present in the sites closest to the beach correlating with the level of recreational activity. UV filters were present at significantly lower concentrations in samples taken further from the beach and in early morning and late evening samples. A number of UV filters were present in higher concentrations in the microlayer samples compared with the corresponding surface water samples. A number of UV filters were detected in seawater collected both at coral depth and the surface, although at much lower concentrations compared to beach samples. This study provides insight as to the environmentally relevant concentrations in seawater samples at beaches and coral reefs in the Florida Keys. Furthermore, the spatial and temporal assessments provide data that are essential for developing probabilistic marine exposure models and environmental risk assessments.

**2.06.P-Mo067 Right-Sizing UV Filter Exposure Estimates - A Critical Need**

*Nikki Maples-Reynolds[1], Scott Dyer[1], Brenna Kent[1], Colleen Roy[1], Raghu Vamshi[1], **W. Martin Williams**[1], Todd Gouin[2], Nicola Hefner[3], Eva Klingelmann[4], Sascha Pawlowski[5] and Amelie Ott[6], (1) Waterborne Environmental, Inc., (2) TG Environmental Research, United Kingdom, (3) DSM, (4) Symrise, Germany, (5) GBP/RA, BASF SE, Germany, (6) Cosmetics Europe, Belgium*

Organic ultraviolet (UV) filters in personal care sunscreen products are vital in preventing skin cancer by absorbing harmful UV-A and UV-B light. Several UV filter chemicals have been associated with potential negative effects in aquatic organisms (e.g., algae, mussels, sea urchins, fish, and corals). UV filters have become a potential contaminant of concern in aquatic systems; however, robust, and high-quality monitoring data, illustrating the actual exposure situation, is limited to date. Environmental exposure information is crucial to conducting environmental risk assessments. The development of mathematical environmental modeling allows estimations of chemicals of concern. Building on a conceptual model of UV filter release into the aquatic environment, a tiered environmental modeling framework for estimating environmental exposure to fresh and marine waters was developed. The framework will enable the prediction of environmental concentrations based on the complexities of exposure scenarios that exist via UV filter, sunscreen, recreational use. The modeling tiers range from simple conservative screening assessment approaches to progressively more sophisticated 2- and 3-D circulation models. Each tier model was selected from open source, publicly available models for transparency with the regulatory community. Tier 0 consists of a simple dilution-based model. Tier 1 is SimpleBox 4.0 a nested multi-media mass balance environmental fate model of the Mackay level III/IV type (fugacity model). Tier 2 is comprised of two three-dimensional models. The first Tier 2 model is Marine Antifoulant Model to Predict Environmental Concentration (MAMPEC) 3.0. The second Tier 2 model is CozMo-POP-2, a mechanistic, dynamic state model describing a drainage basin corresponding to a marine coastline. The final Tier 3 model is 2-D or 3-D circulation model such as, United States Environmental Protection Agency's, Environmental Fluid Dynamics Code (EFDC). Exposure results from each tier will be compared to sites with analytical monitoring data in Europe (Marseille, France) and the United States (Hawaii). Both pros and cons for each tiered approach in estimating environmental concentrations will be discussed using the monitored data. The ultimate objective is to develop scenarios that represent a reasonable range of situations to evaluate potential ecological risks of existing and future UV sunscreen formulations.

**2.06.P-Mo068 Sunscreen Formulation is a Crucial Factor for Modeling Rinse-off of Ultraviolet Filters in Seawater**

*Jennifer K. Saxe[1], Larry A. Mullins[2], Randy L. Jones[2], Alan Lewis Jr.[2], Frank Sun[3] and **Kurt Reynertson**[3], (1) EcoSafety & Sustainability Inc., (2 )Battelle Memorial Institute, (3) Johnson & Johnson Consumer, Inc.*

Significant research and regulatory attention have focused on the potential for some ultraviolet filters (UVFs) in sunscreen to rinse off from beachgoers' skin into seawater leading to exposure to sea life, especially coral reefs. Modeling – with corroboration from the limited available monitoring data – is needed to understand the potential for sea life exposure to UVFs from sunscreens. To support this exposure modeling, we applied sunscreen to porcine skin followed by saltwater rinsing to simulate ocean bathing and measured UVFs in rinsates. Results showed that for the five organic UVFs (avobenzone, homosalate, octisalate, octocrylene, and oxybenzone) and two inorganic UVFs (zinc oxide and titanium dioxide) tested, the overall composition of the sunscreen is a significant factor in the amount of UVF rinsed from skin. Among the product forms tested, anhydrous and reverse emulsions (*i.e.*, oil continuous phase with dispersed polar internal phase) were better for retaining UVFs on skin than the more common oil-in-water emulsions. This research supports the selection of appropriate rinse-off estimates for exposure modeling and product design choices that can minimize potential UVF exposures in sea water.

**2.06.P-Mo069 Adjusting Sampling Strategies to Account for Environmental Partitioning of UV Filters**
***Stefanie Landeweer*** *and Piero Gardinali, Florida International University*
Organic ultraviolet (UV) filter compounds are the subject of increasing environmental scrutiny due to reported harmful effects to marine life. Knowing the environmental prevalence of these compounds is critical to understanding the potential for organisms to be exposed. However, traditional sampling methods may be giving a limited picture of the environmental prevalence of UV filter compounds. Oxybenzone and avobenzone, two common commercial UV filters, have log octanol-water partition coefficient ($logK_{OW}$) values of 3.8 and 4.5, respectively, indicating a hydrophobic nature. Therefore, it is likely that these compounds will tend to partition towards surfaces rather than remaining dissolved in the water column. This possible behavior in the aquatic systems raises the necessity of using specific necessitates the use of sampling methods that account for the compounds' partitioning to gain an in-depth understanding of UV filters in the environment. Two possible UV filter sinks were investigated in this experiment: the water surface microlayer and anthropogenic objects such as boats and buoys. Both were compared to a traditional sub-surface grab sample. The area of the study was Oleta River State Park, located in the suburbs of Miami along the shore of Biscayne Bay, due to its popularity with visitors and calm semi-enclosed waters. Studies carried out by diffusing oxybenzone from one gram of a commercial sunscreen formulation through a permeable PDMS membrane into artificial seawater resulted in an equilibrium concentration of 1.78 mg/L. However, environmental data from Biscayne Bay shows a maximum reported oxybenzone concentration of 58 ng/L even in areas with many nearby bathers in the water. Therefore, it was hypothesized that the majority of oxybenzone in the ocean was partitioning out of the water column and into other areas, such as the surface microlayer. Preliminary investigation carried out in a nearby area of Biscayne Bay supports this hypothesis with an average concentration of oxybenzone at the surface found to be ten times higher than the average oxybenzone concentration in sub-surface samples collected at the same time and location. The significance of this study lies in the demonstration of which sampling techniques are needed to provide a better understanding about the UV filters' distribution in the environment. With knowledge of the environmental partitioning of these compounds their true prevalence and adverse effects can be more fully known.

**2.06.P-Mo070 Toxicity of the UV filter octocrylene to the scleractinian coral Acropora cervicornis**
***Carys Louise Mitchelmore***[1]*, Andrew Heyes*[1]*, Michael Gonsior*[1]*, Iain Davies*[2]*, Ellen R. Skelton*[3] *and Dorothy-Ellen Abigail Renegar*[3]*, (1)University of Maryland Center for Environmental Science, (2) PCPC (Personal Care Products Council), (3) Nova Southeastern University*
Ecotoxicological studies have shown variable responses in several coral species following exposure to organic UV filters. Octocrylene has been investigated in a number of aquatic organisms, however, limited studies and effects on corals have been reported to date. To address this knowledge gap we conducted range-finding and definitive acute (i.e. 96 hour) and chronic (i.e. 21 day) toxicity tests, based on standard US EPA and OECD guidelines, with the Atlantic staghorn coral *Acropora cervicornis*. Multiple biological endpoints were employed to assess the response of *A. cervicornis* compared to other corals, invertebrate and algal species, including those pertinent to risk assessments (i.e. mortality and growth) along with other coral specific assessments, such as visual bleaching, coral condition (i.e. mucus production, tissue swelling and/or thinning), polyp retraction and

photosynthetic efficiency. Acute toxicity was not observed with octocrylene at concentrations in the mg/L range and exceeding solubility estimates. Chronic toxicity tests were conducted using concentrations below estimated solubility and at levels measured in seawater (i.e. 0.05 – 4.05 µg/L nominal). Following 21-days exposure no statistically-significant effects were observed for visual bleaching, photosynthetic efficiency or coral condition at any concentration or time point. At 21-days only a statistically-significant reduction in growth compared to controls was observed at the highest concentration. Chronic toxicity in *A. cervicornis* is similar to that observed with the invertebrate standard toxicity test species *Daphnia magna*. Further studies are required to investigate the impact of octocrylene on a range of morphologically diverse and geographically distributed coral species using both adult and early-life stages in addition to the extent of bioaccumulation and mechanism of action of octocrylene in coral species.

**2.06.P-Mo071 Nephrotoxic potentials of major organic UV filters (AVB, BP-3, OC, OMC) in zebrafish of different life stages**
***Ba Reum Kwon****, Ah-Reum Jo, Jung Pyo Lee, Jeongwhan Lee, Juyeon Choi, Yeonju Nam, Eun-Jin Kim, Gowoon Lee, Inae Lee and Kyungho Choi, Seoul National University, Korea, Republic of (South)*
Organic UV filters are widely used for skin protection not only in sunscreen but also in various personal care products such as cosmetics, and hair products. Growing laboratory and epidemiologic evidence raised concerns of potential health impacts of UV filters, however information is mostly limited to reproduction/sex hormones or thyroid endocrine systems. The present study aims to investigate nephrotoxic potentials of four major organic UV filters, i.e., avobenzone (AVB), benzophenone-3 (BP-3), octocrylene (OC), and octinoxate (OMC) using zebrafish (ZF) model of different life stages. Different concentrations of test UV filters were applied to embryo-larval (for 5 days until 5 days post fertilization, dpf), early-life (30 dpf), and adult stage (>6 months old, 21 d exposure) of ZF. Following exposure, embryo-larval and juvenile fish were examined for proteinuria, a widely used indicator for kidney function. Moreover, genes related to nephrogenesis and kidney injury were quantified in whole body of embryo-larval and juvenile ZF, and kidney tissues in adult fish. Significant induction of proteinuria level in both embryo-larval and juvenile ZF was observed following exposure to AVB, BP-3 and OMC. Regulatory changes of key genes responsible for kidney structure and function showed similar profiles, suggesting both fish of early and adult life stages could be influenced by UV filter exposure. For example, genes of ZF glomerulus (*wt1a*, *podocin*, *nephrin*, *cdh17*) and tubules (*sim1a*) were significantly down-regulated; and *kim-1* gene which is expected to up-regulate upon injury, was significantly up-regulated in embryo-larval, juvenile and adult zebrafish after UV filter exposure. Overall, our observation clearly demonstrate that exposure to widely used sunscreen chemicals could impair kidney function possibly through compromising the genes that encode key proteins that are essential for maintaining the structure and function of the kidneys. Further studies for potential key events linking transcriptional changes to kidney function are warranted.

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**2.06.V Detection, Toxicity and Environmental Risk of UV filters in Aquatic Ecosystems**

**2.06.V-01 Spatial Distribution and Partitioning of UV Absorbents in the Surface Water of the St. Lawrence River and Estuary in Canada**
***Amina Ben Chaaben**[1], Mathieu Babin[2], Frank Wania[3], Hayley Hung[4], Magali Houde[4], Liisa Jantunen[4], Huixiang Xie[2] and Zhe Lu[2], (1) University of Quebec, Rimouski, Canada, (2) Institut des Sciences de la Mer de Rimouski, Canada, (3) University of Toronto Scarborough, Canada, (4) Environment and Climate Change Canada*
Organic UV filters (UVFs) and benzotriazole UV stabilizers (BZT-UVs) are two groups of UV absorbents

(UVAs) of emerging environmental concern. They have been detected in tissues of fish (e.g., northern pike (*Esox lucius*)) from the St. Lawrence River and beluga whales (*Delphinapterus leucas*) from the St. Lawrence Estuary in Canada, indicating the accumulation of these contaminants in aquatic organisms. However, the concentrations, distributions, and speciation (e.g., partitioning between aqueous and solid phases) of UVAs, important factors influencing their fate and uptake by aquatic organisms, are largely unknown in the water column of the St. Lawrence River and Estuary (SLRE). The current study aims to elucidate (1) the spatial distributions of UVAs including UVFs and BZT-UVs in surface water of the SLRE; (2) the levels and chemical profiles of these contaminants between coastal and offshore sites; and (3) their field-based partitioning between water and suspended particulate matter. To this end, analytical methods based on solid phase extraction and gas chromatography-tandem mass spectrometry have been developed to measure these contaminants in the aqueous dissolved phase and the particulate matter of water samples. These methods are currently being used to analyze surface water samples collected from 48 coastal sites and 15 offshore sites in the SLRE in 2019 and 2020. Preliminary results for the aqueous dissolved phase showed the detection of six UVFs and eight BZT-UVs in five studied sites in the SLRE. For the 5 studied sites, the concentrations of total UVFs (up to 742 ± 251 ng/L; mean±SD) were 3-14 times higher than total BZT-UVs (up to 53 ± 30 ng/L). The water samples from the Quebec City site showed the highest levels for total UVFs, possibly suggesting that Quebec City is a source of UVFs to the estuary. For total BZT-UVs, there were no clear spatial patterns among the five studied sites. Benzophenone (BP) was the most prevalent UVF, followed by 2-ethylhexyl salicylate (EHS) and 3,3,5-trimethylcyclohexyl salicylate (HMS). For BZT-UVs, 2-(2H-benzotriazol-2-yl)-p-cresol (UVP) was detected at the highest levels, followed by 2-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethyl butyl)phenol (UV329) and 2-*tert*-butyl-6-(5-chloro-2H-benzotriazol-2-yl)-4-methylphenol (UV326). This study revealed the presence of UVFs and BZT-UVs in the surface water of SLRE, implying that aquatic organisms in the SLRE may be exposed to these contaminants via surface water.

**2.06.V-02 Developing Standard Ecotoxicity Tests on Scleractinian Corals for Ultraviolet (UV) filters and Related Chemicals**

***Valentina Di Mauro**[1]*, *Ingo Miller[1], Matthias Kellermann[1], David Brefeld[1], Samuel Nietzer[1], Mareen Moeller[1], Elham Kamyab[1], Laura Henriette Lütjens[2], Sascha Pawlowski[2], Mechtild Petersen-Thiery[3] and Peter Schupp[1], (1) University of Oldenburg, ICBM, Germany, (2) BASF SE, Germany, (3)BASF Personal Care & Nutrition GmbH, Germany*

Coral reefs are marine hotspots of biodiversity and provide vital ecosystem services. Due to climate change and ocean acidification, reef building corals are declining at a dramatic pace. Not only global but also local stressors impact coral health. UV filters used in personal care products such as sunscreens are considered to be among those substances due to their direct release into the coastal marine environment. Some research findings indicate a possible impact on corals after exposure to certain UV filters such as bleaching or even mortality, whereas other scientific publications did not. Thus, the existing results are considered as controversial due to experimental deficiencies and require further investigation on corals. Using standardised and validated testing protocols would certainly help for a sound and scientifically based conclusion and will also support regulatory decision-making processes. Consequently, we currently develop short and long-term testing methods on corals of different life stages (e.g., larvae and adult stage) suitable for validation and standardisation within the DIN, ISO and/or OECD framework.

So far, our developed ecotoxicity assays for chemicals including those that are poorly water soluble (i.e., UV filters) on corals of different life stages showed reliable test results. As an example, our coral larval settlement assay presented an inverse correlation of the endpoints mortality and metamorphosis, whereas our adult coral bleaching assay displayed a positive correlation of the endpoint bleaching in a concentration-response relationship. As a next step, additional pre-validation work in collaboration with additional laboratories is needed to assess the interlab variability and reproducibility of our testing method and results.

**2.06.V-03 Trophodynamics of Industrial Antioxidants, UV Absorbents and Polyhalogenated Carbazoles in the Food Web of the Endangered St. Lawrence Estuary Beluga Population**

***Alexis Eric Adrien Trinquet*[1]**, *Alexis Agathine*[1], *Mathieu Babin*[1], *Jonathan Verreault*[2], *Véronique Lesage*[3], *Jory Cabrol*[3], *Stephane Lair*[4], *Gerald Tetreault*[5] *and Zhe Lu*[1], *(1) Institut des Sciences de la Mer de Rimouski, Canada, (2 )University of Quebec at Montreal, Canada, (3) Fisheries and Oceans Canada, Canada, (4)University of Montreal, Canada, (5) Environment and Climate Change Canada*

Industrial antioxidants (IAs), UV absorbents (UVAs) and polyhalogenated carbazoles (PHCZs) are contaminants of emerging concern in marine environment that may cause adverse health effects in organisms. A recent study from our laboratory detected IAs and UVAs in the blubber and liver samples collected from beluga carcasses of the St. Lawrence Estuary (SLE), a population that is considered endangered in Canada. However, the trophodynamics of these contaminants in the food web is currently unknown. To fill this knowledge gap, tissues were collected from 16 known and potential prey of SLE beluga in 2019 and 2020 to investigate the biomagnification patterns of selected IAs, UVAs, and PHCZs in the SLE beluga's food web. We hypothesized that PHCZs have greater biomagnification potential than IAs and UVAs. Carbon sources and trophic levels of all beluga prey items were determined using stable isotopes to investigate relationships between prey ecology and contaminant concentrations to identify the most probable dietary sources for these contaminants. Contaminants were analyzed using a gas chromatograph coupled to a triple quadrupole mass spectrometer (GC-MS/MS). Our preliminary results indicate the presence of one PHCZ, two IAs and 6 UVAs in winter flounder (*Pseudopleuronectes americanus*) ($n$=5) and/or northern shrimp (*Pandalus borealis) (n=5)*. The predominant UVAs in winter flounder were OC (2-Ethylhexyl 2-cyano-3,3-diphenylacrylate) (29.4 ng.g$^{-1}$ ww) and HMS (3,3,5-Trimethylcyclohexyl salicylate) (16.11 ng.g$^{-1}$ ww) while the major UVAs in northern shrimp were EHMC (Ethylhexyl methoxycinnamate) (4.98 ng.g$^{-1}$ (ww)) and UV320 (2-Benzotriazole-2-yl-4,6-di-*tert*-butylphenol) (3.47 ng.g$^{-1}$ (ww)). Analysis for the remaining samples is underway. This study will contribute to a better understanding of the environmental fate of these contaminants in the SLE food web.

**2.06.V-04 The UV-filter 3-Benzophenone Does Not Cause Oxidative Stress in Octopus maya (Voss y Solís-Ramírez, 1966) During Embryonic Development**

***Gissela Moreno Ortiz***, *Universidad Nacional Autónoma de México*

3-Benzophenone (3-BP) is a widely used ultraviolet (UV) filter added to personal care products and other consumer goods, however, it potentially harms humans, wildlife, and ecosystems. It has already been shown that 3-BP could be a possible contributor to coral reef bleaching, act as an endocrine disruptor, and increase the production of reactive oxygen species in fish. However, there is no ecotoxicological information on exposure to 3-BP in octopus. *Octopus maya* is an endemic specie from Yucatan Peninsula in Mexico and represents the most important fishery in the area with an annual production reaching 30,000 Tons.

This study investigated the effect of 3-BP on the antioxidant enzymes activities superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), and total glutathione concentration (GSH), and the oxidative damage indicators: protein carboxylation (PO) and lipoperoxidation (LPO) during *Octopus maya* embryo development. At the same time, the routine metabolic rate (RMR) of embryos was measured and used to evaluate the physiological condition of the embryos exposed to 3-BP. The experiment was designed considering relevant environmental concentrations of 3-BP of 5, 25, 50, and 500 µg/L and two control groups: embryos in natural seawater and exposed to DMSO (0.01%) used as a solvent carrier of 3-BP.

Embryos were separated in four critical embryo developmental stages: blastula (I-IX), organogenesis (stages X-XIII), activation (stages XIV-XVI), and growth (stages XVII-XIX) in an attempt to evaluate if 3-BP could be affecting a particular stage. Results showed an increment in the activity of antioxidant enzymes and the concentration of GSH and LPO along embryo development. At the same time, a decrease in PO during development was observed indicating that antioxidant defense mechanisms in the embryo worked to neutralize the production of carbonylated proteins. Also, an increment of RMR of embryos was observed with the

maximum metabolic activity in the activation phase and it could be related to the systemic and branchial hearts beginning to beat. There was no effect of 3-BP on antioxidant defense mechanisms and respiratory metabolism suggesting that embryonic chorion is an efficient barrier against exogenous pollutants. Also, in this early life stage, metabolizing enzymes could be not fully active.

## 2.07 Fate and Effects of Chemicals from Stormwater Runoff

### 2.07.T-01 Developing Novel Indicators of Sewage Collection Systems for Source Tracking During Storm Events

***Kenneth Schiff*** *and Joshua Steele, Southern California Coastal Water Research Project (SCCWRP)*

Despite the known human and aquatic life risks associated with raw sewage, source tracking tools for sewage in urban runoff remain challenging. Many chemical source tracking tools usually lack sensitivity to detect low-levels of sewage, microbial tools typically lack the specificity for sewage, and many novel sensors are inhibited by stormwater matrix. Source tracking tools for sewage are particularly challenging in wet weather when the myriad of pollutant sources are dynamically mixing. Finding sensitive and reliable source tracking tools are particularly important in southern California because sanitary sewer collection systems and municipal storm drainage systems are separate (e.g., no combined sewer overflows). The aim of this study was to develop unique markers of the public sanitary sewer collection system utilizing the microbial community profiles of biofilms that line the inside of pipes (sewage biofilms). If sewage biofilms are quantified in stormwater runoff, then the sewer collection system is somehow leaking and entering the storm drain system. The unique sewage biofilm marker development followed four major steps: 1) building a prototype sampling device for collecting biofilms and refining high-throughput DNA sequencing technology; 2) ensuring the microbial community profiles from sewer biofilms were consistent among pipes of different ages, materials, locations, and season; 3) confirming microbial community profiles from sewer biofilms were distinctly and consistently different from storm drain biofilms, and; 4) quantifying sewer biofilms from the storm drain system during wet weather. After sampling more than 60 sites across multiple seasons over two years, microbial communities in sewage biofilms were extremely consistent yet distinctly different than storm drain biofilms based on non-metric multi-dimensional scaling (nMDS). After sampling 13 sites over two wet seasons across a 900 sq km urban watershed, sewage biofilms were quantified during every storm at over 90% of the storm drain sites. However, concentrations of sewer biofilms in storm drains were consistently dilute, typically less than 0.1% relative to raw sewage. New research is now focusing on quantifying exfiltration from the 5,000 km of sanitary sewer collection system pipe infrastructure.

### 2.07.T-02 The State of the Practice for Industrial Stormwater Pollutant Source Identification and Control

***Stacey Lee Isaac****, Brandon Steets and Jared Ervin, Geosyntec Consultants, Inc.*

Stormwater discharges from industrial facilities are often subject to low pollutant benchmarks, which can result in high costs for treatment and impact business and operations. However, state and federal industrial stormwater regulations only require control of pollutants associated with industrial activity and materials. Therefore, it is critical to distinguish whether stormwater pollutants exceeding benchmarks are exclusively from non-industrial sources (e.g., roof materials, asphalt weathering, vehicle tires and brake pads, atmospheric deposition) and/or background sources (e.g., natural soils). This can be done through hypothesis-driven forensic sampling investigations to support Non-Industrial Source Demonstrations (NISDs) or implementation of source control Best Management Practices (BMPs) as a low cost means to achieve benchmarks. If treatment is needed, costs may be reduced by minimizing the drainage area requiring treatment. This can be accomplished by distinguishing stormwater impacted by industrial activities vs non-industrial areas, as well as through spatial sampling to delineate specific areas that exceed benchmarks.

Multiple forensic approaches may be used to determine the sources of stormwater pollutants at industrial facilitates. For example, for highly particulate-form pollutants, particulate strengths (i.e., pollutant particulate mass per mass of suspended solids) are useful for identifying sources by comparison to solids sampling results (e.g., of background or impacted soils, pavement solids, atmospheric deposition solids, wildfire ash). Other approaches for source determination include analyses of metals ratios, congener analysis/fingerprinting for dioxins, and isotope analysis for lead.

This presentation will explore approaches that have been used to identify the source of pollutants – both activities/materials and spatial areas – to stormwater runoff at industrial facilities. Methods for investigating the source of pollutants, treatment controls implemented, and resulting performance of treatment approaches will be discussed. Source investigation and long-term BMP performance monitoring results will be shared from an industrial site in southern California under strict numeric effluent limits for a variety of contaminants, including radionuclides, organics, and metals. Additionally, an approach for performing a NISD will be discussed.

**2.07.T-03 Exposures and Potential Ecological Effects of Chemical Mixtures in Unregulated Stormwater**
***Kelly L. Smalling***[1]*, Paul M. Bradley*[1]*, Sara Breitmeyer*[1]*, Kristen M. Romanok*[1]*, Jason R. Masoner*[1]*, Dana Kolpin*[1]*, Michelle L. Hladik*[1] *and John Bunnell*[2]*, (1) U.S. Geological Survey, (2) New Jersey Pinelands Commission*
Habitat loss, fragmentation, and degradation due to increased urban development can negatively affect the ecological function of geographically isolated wetlands and lead to a reduction in local and regional biodiversity. Urban stormwater basins – which are designed to mimic natural wetlands – are built to retain runoff and promote groundwater recharge but can also function as ecological traps for aquatic organisms. Several studies were designed to help better understand the potential risks of stormwater-related contaminant mixtures to biodiversity in urban landscapes and to better conserve stormwater basins as habitat for native fish and wildlife. To understand contaminant exposure broadly, we conducted a nationwide study in 2016-2017 of 438 organic and 62 inorganic chemicals in urban stormwater samples from 21 sites in 17 states. We also conducted several smaller place-based studies in 2016–2020 designed to assess the relative ecological functionality and integrity of stormwater basins by comparing hydrologic conditions, water quality, and wetland assemblages (e.g., plants and anurans). Results from these studies support the notion that stormwater run-off and receiving basins are a source of organic and inorganic contaminants mixtures known or suspected to pose an environmental health concern. These studies broadly inform resource conservation strategies for communities with diverse habitat requirements, particularly in areas where conservation and development are competing priorities. If managed properly (e.g., retaining a similar functionality to natural wetlands), stormwater basins can provide beneficial habitat for aquatic organisms, particularly in fragmented landscapes where water is scarce, and habitat is limited.

**2.07.T-04 The Effects of Dissolved Organic Carbon (DOC) on Metal (loid) Dynamics in Stormwater Bioretention Bed**
***Abdullah Al-Amin****, Robert J. Ryan and Erica R. McKenzie, Temple University*
Dissolved organic carbon (DOC) is heterogeneous and consists of various molecular weight compounds with a range of functional groups (carboxyl, carbonyl, phenol, aryl, and amide). Dissolved organic carbon (DOC) has the capacity to form complexes with metals (loids) which in turn influences metals (loids) fate and transport. Stormwater runoff can contain DOC and metals (loids), and other contaminants, which are introduced to the stormwater management practice (SMP) which consists of soil media with some sorption capacity for contaminants. This study focused on the effects of DOC on metal (loid) dynamics in the horizontal and vertical transects of the SMP bioretention bed.

Samples were collected in longitudinal and vertical transects of the SMP bioretention bed. A batch study was conducted to evaluate the effect of DOC (0, 15, and 50 mg-C/L) on the dynamics of ten metals (loids) [iron, manganese, cobalt, cadmium, copper, zinc, arsenic, tin, antimony, and lead]; additionally, sequential extractions were conducted to determine exchangeable (bioavailable), carbonate-bound, mineral-bound, and organic-matter-bound fractions of these metals (loids).

Leachate metal (loid) concentration tended to increase with increasing DOC concentration in the longitudinal and vertical transects. Metal (loid) concentration decreased with increasing distance from the SMP inlet, regardless of DOC concentration, which may be caused by incoming suspended solid accumulating near the inlet. Metal (loid) leachate concentration decreased with increasing depth in the vertical transects. Extracted metal (loid) revealed a general trend for cumulated metal (loid) fractions: mineral bound ~ organic matter bound > carbonate bound > exchangeable. No significant trend was found for the effect of DOC on different extracted fractions. These results indicate that there are clear spatial patterns, with greater leachable metals (loids) near the inlet and that DOC could increase leachability, but that vertical mobility of metals (loids) is limited based on four years of SMP use. These dynamic interactions between metals (loids) and DOC will enable better designing the stormwater bioretention bed and infiltration media for effectively intercepting the contaminants.

**2.07.T-05 Vegetative Buffer Strips for Reducing Antibiotic Transport in Surface Runoff: Effects of Buffer Width and Vegetation**

***Adam Moody****, U.S. Geological Survey*

Multi-species of vegetative buffer strips (VBS) have been recommended as an effective approach to mitigate agrochemical transport in surface runoff derived from agronomic operations, while at the same time offering a broader range of long-term ecological and environmental benefits. However, the effectiveness of VBS designs and species composition on reducing veterinary antibiotic (VA) transport from surface applied manure has not been well documented for runoff prone soils. A study was conducted to assess effectiveness of three VBS designs and one continuous cultivated fallow control for reducing surface transport of the environmentally mobile VAs, sulfamethazine (SMZ) and lincomycin (LIN), in claypan soils. The three VBS designs included: (i) tall fescue (TF), (ii) tall fescue with a switchgrass hedge barrier (Hedge+TF), and (iii) native vegetation consisting of warm-season grasses (Native). A rotating-boom rainfall simulator was used to create uniform antecedent soil water content and to generate runoff. The VBS buffer width impacted VA loads much more than vegetation treatment. Results showed that the VBS treatments were very successful at achieving sediment load reductions (82 – 90%) but had limited success at reducing dissolved or sediment-bound VA loads. Dissolved-phase VA loads were significantly reduced by only TF (59% for SMZ and 61% for LIN) as the grass treatments had greater runoff compared to the control. Buffer width was a more significant factor for dissolved-phase loads of LIN, with significant load reductions occurring as width increased, while SMZ had similar load reductions at each width. However, VBS treatments significantly reduced total (dissolved-phase + sediment-bound) SMZ and LIN loads in surface runoff by 29 to 62% with TF as the overall most effective treatment at reducing total VA loads (62% for SMZ and 61% for LIN). Sediment-bound loads of both VAs were strongly affected by buffer width, but not by vegetation treatment. The results showed that grass treatment had less effect on VA loads than buffer width, and the grass treatments tested here were less effective at reducing SMZ and LIN loads in surface runoff than has been demonstrated for other contaminants, such as sediment, nutrients, and herbicides. Effectively reducing loads of LIN and SMZ in runoff, especially via dissolved-phase transport, would require wider buffer widths and more land out of production than for other agricultural contaminants.

**2.07.T-06 Antiozonants: Adding 6PPD to the 6PPD-quinone Story.**

***Million Woudneh****, Bharat Chandramouli and Coreen Hamilton, SGS AXYS Analytical Services Ltd., Canada*

Ambient ozone attack on the common tire antiozonant, 6PPD, produces the toxic oxidation product 6PPD-quinone. The acute toxicity of 6PPD-quinone to coho salmon and its entry into water sources through road runoff has raised public concern. Some of the first 6PPD-quinone environmental monitoring results have started to appear in the open literature. However, due to its extreme instability, environmental levels are not available for 6PPD, and measuring only 6PPD-quinone, without measuring the unreacted 6PPD, represents an underestimation of the toxic potential of a water sample. In this study, we present environmental levels of 6PPD and 6PPD-quinone in surface water runoff. The results showed that 94% and 88% of the aqueous samples showed detected levels of 6PPD-quinone, and 6PPD respectively. The measured concentrations ranged from 0-740 ng/L for 6PPD-quinone and 0-5100 ng/L for 6PPD. On average, the concentration of 6PPD was 3.5x that of the 6PPD-quinone and demonstrated the importance of measuring 6PPD together with 6PPD-quinone. The measured values for both analytes appeared to show a relationship to the sampling season, wet and dry. The highest concentrations were detected in the wet season, and the lowest concentrations were detected in the dry season. This study also highlights the difficulties associated with measuring the unstable 6PPD and ways to partially stabilize it in the sample and during the analytical procedure. To the best of our knowledge, this study represents the first reporting of environmental levels for 6PPD with 6PPD-quinone. The results reported in this study can be used by regulators, laboratories, and researchers involved in antiozonant study in the environment.

**2.07.T-07 Toxicity of 6PPD-quinone Among Estuarine Fishes During Development**

***Kerri Lynn Ackerly****, Kathleen Roark, Andrew Esbaugh and Kristin Nielsen, The University of Texas at Austin*

Annual large-scale fish kills after storms are a serious issue for salmon populations migrating through highly urbanized watersheds in the Pacific Northwest region of the United States. Recently, these mass die-offs were linked to tire wear particles (TWPs) left on roadways, which are washed into nearby surface waters with stormwater runoff. The lethality of these TWPs have been attributed to 6PPD-quinone (a quinone byproduct of the ubiquitous tire antiozonant 6PPD), which has a median lethal concentration of < 1-µg/L for select species of salmonids. However, there remains a paucity of data describing the differential toxicity of this compound across species and ontogenies, or its sub-lethal effects during early life stages. Additionally, no toxicity values have been developed for estuarine fish species exposed to 6PPD-quinone. Filling this data gap is particularly significant because estuaries receiving inflows from highly urbanized watersheds are especially vulnerable to TWPs. To close these data gaps, we (1) determined the toxicity of 6PPD-quinone to both model (e.g., sheepshead minnow [*Cyprinodon variegatus*]) and non-model (red drum [*Sciaenops ocellatus*], southern flounder [*Paralichthys lethostigma*]) estuarine fishes (2) examined the relative sensitivities of early life stages within a single species, and (3) derived sub-lethal toxicity values for early life stage fish. Results indicate that the toxicity of 6PPD-quinone varies demonstrably across species and ontogeny, with toxicity values spanning several orders of magnitude in some species. These data are a significant contribution towards advancing our knowledge of this newly identified and likely pervasive environmental toxicant.

**2.07.T-08 Understanding the Environmental Occurrence and Fate of the Tire Leachate Transformation Product 6PPD-Quinone**

***Rachael Lane****, Paul M. Bradley, Dana Kolpin and Kelly L. Smalling, U.S. Geological Survey*

Recent studies have linked the urban runoff mortality syndrome in coho salmon with the rubber antioxidant transformation product, N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-Quinone). The widespread use of rubber in products such as tires and the documented harmful environmental effect necessitates improved understanding of the occurrence, fate, and bioavailability of 6PPD-Quinone. To explore environmental prevalence, the U.S. Geological Survey (USGS) Organic Geochemistry Research Laboratory analyzed by liquid chromatography tandem mass spectrometry (LC/MS/MS) a variety of filtered water samples including drinking water, urban stormwater, surface waters, urban-impacted surface waters, and wastewater-impacted surface waters from more than 20 locations across the United States including sites from the USGS National Water Quality Network (NWQN). These samples were collected during 2022 or selected from a frozen archive of samples to represent a multiyear timeframe and disparate locations. While it has been observed that the frequency of 6PPD-Quinone detections and concentrations increase during urban stormwater runoff, these events were not preferentially selected from the archived samples. Additionally, adsorption experiments with soils and bed sediments were conducted and the partitioning between solids and water represents an early step in understanding the fate and bioavailability of 6PPD-Quinone to flora and fauna in the environment.

## 2.07.P Fate and Effects of Chemicals from Stormwater Runoff

### 2.07.P-We006 Acute toxicity of 6PPD and its transformation products to larval mayflies (Neocloeon triangulifer) and juvenile freshwater mussels (Lampsilis siliquoidea)

***David J. Soucek***[1], *Rebecca Dorman*[1], *Jeff A. Steevens*[1], *Viviane Yargeau*[2], *Daniel Furlong*[2], *Erin Bennett*[3] *and Chris D. Metcalfe*[3], *(1) U.S. Geological Survey, (2) McGill University, Canada, (3) Trent University, Canada*

The tire rubber antioxidant, N-(1,3-dimethylbutyl)-N' -phenyl-p-phenylenediamine, or 6PPD and its transformation products (TP), including 6PPD-quinone, have previously been shown to be acutely toxic to several freshwater species.  Some salmonids, including coho salmon (*Oncorhynchus kisutch*), rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) are particularly sensitive to 6PPD-quinone, while previous studies demonstrated that several other fish species and two invertebrates showed no responses to exposures at or near the solubility limit of this compound.  6PPD is less acutely toxic than the quinone TP to coho salmon and several other aquatic species, with effects typically observed at concentrations in the 100-1000 µg/L range for this more water-soluble compound.  We investigated the acute toxicity of 6PPD, 6PPD-quinone and a crude product prepared by ozonation of 6PPD to early instars of the mayfly, *Neocloeon triangulifer*, and to juveniles of "fatmucket" mussels, *Lampsilis siliquoidea*.  All test solutions were prepared with a small volume (≤0.2 mL/L) of acetone as a carrier solvent.  For 6PPD, the 96-h LC50 based on nominal concentrations for the mayfly of 294 µg/L was similar to LC50s observed in previous studies for *Daphnia magna* and *Hyalella azteca*, while the 96-h EC50 for mussels of 1,240 µg/L (nominal concentration) was higher than any other lethal concentration reported in the literature, to our knowledge.  The 6PPD-quinone caused no mortality to either the mayfly or the mussel test species at the approximate water solubility limit of ~90 µg/L.  The crude product of 6PPD ozonation was less toxic than 6PPD for both test species, with 96-h LC/EC50s of 477 and 1,348 µg/L (nominal concentrations based on the original amount of 6PPD before ozonation) for the mayflies and mussels, respectively.  Analytical studies using LC-HRMS are underway to verify exposure concentrations and to determine whether known and unknown TPs were present in the exposure solutions. Studies are currently underway to investigate whether there are changes in the toxicity of 6PPD to the mayfly when solutions are aged under light and dark conditions and to determine analytically the patterns of TPs generated under these different light regimes.  Our results will contribute to a better understanding of the toxicity of these tire wear compounds under a range of environmental conditions.

### 2.07.P-We007 Use of Combined Modeling Approach to Evaluate Transportation-Related Stormwater and its Potential Impacts to Sediment

***Jillian DeMars***, *Philip Spadaro and Jason Dittman, TIG Environmental*

In an urban waterway cleanup setting, it is essential to understand and quantify the potential impacts of continued inputs (such as combined sewer overflows, stormwater, or direct process discharges) from the waterway drainage basin to identify contaminant sources and mitigate any potential for recontamination of the planned remedy. Sediment investigation, remediation and cost allocation is a very complex and lengthy process. Distinguishing in-water, waterfront and upland sources and attributing remediation costs for those sources is a task which has taken decades to accomplish. Although land uses may vary greatly, major transportation (roadways, bridges, dedicated outfalls and supporting maintenance facilities) is a commonality amongst urban waterway drainage basins.

This presentation will discuss a multi-step modeling approach to characterizing transportation-related stormwater and quantifying its potential impacts to surface and subsurface sediment. This approach utilizes TIGSED, a tool based on the SEDCAM model, which is uniquely adaptable and can be modified to account for multiple source areas, operating periods, release mechanisms, and discharge points. The model ultimately can be used to answer two key questions: (1) will transportation runoff result in some degree of contamination exceeding site thresholds, and (2) what are the cost impacts of these discharges? Specifically, the presentation will elaborate on various features of the model, each of which work together to provide a quantitative basis which a transportation entity can utilize in a private allocation setting or other regulatory context (e.g., EPA, state regulatory agencies). We will discuss the various transportation-related environmental data inputs, key assumptions, and complexities of this multi-step approach and how they may be used to assess the impacts of transportation-related runoff in a river setting.

**2.07.P-We008 Investigation into the fate of 6PPD and 6PPD-quinone in air and aquatic conditions**
***Rory Mumford**, Fate and Metabolism, Smithers ERS Limited, Harrogate, United Kingdom*
Urban Runoff Mortality Syndrome (URMS) is a term coined in the last decade to describe the link between prespawn mortality in coho salmon and impacted stormwater. Recent research pinpoints some of the pollutants that are potentially toxic to species in urban watercourses. A degradation product of 6PPD, 6PPD-quinone, was identified which is assumed to derive from tire and road wear particles (TRWP) and washed into watercourses during storm events. Published data from research groups have shown sensitivity of coho salmon to 6PPD-quinone; mortality can occur within a few hours of exposure. 6PPD is one of the most widely used antiozonants present in tires produced globally. Additionally, the effects on aquatic environments of 6PPD itself have been thoroughly studied, but to this day, degradation products of 6PPD (and its homologues) and their environmental relevance were not widely considered, and 6PPD-quinone has only recently been identified.

The current study was undertaken to further understand the behaviour of 6PPD and 6PPD-quinone once they have entered the aquatic environment. $^{14}C$-radiolabelled 6PPD and 6PPD-quinone were synthesised to aid the tracking of degradation products throughout the study. The hydrolytic behaviour of 6PPD and 6PPD-quinone were studied through the environmentally relevant pH range using the standard OECD TG111 buffers. Because trace elements are known to impact the rates and the routes of degradation in the environment, sterile natural waters were used to assess the influence of alkalinity and hardness. Comparison with active natural water was made to assess the biotic effect on hydrolysis and degradation.

A second element of this study was designed to generate data around the degradation of 6PPD and 6PPD-quinone in air. The radiolabelled compounds were applied as a thin film to the inner surface of glass vessels before sealing. A constant stream of nitrogen, air, or air containing modified levels of ozone was passed over the thin film. Vessels were either kept in the dark or irradiated using simulated sunlight to study the effects of oxygen and ozone in the presence of light on the degradation of 6PPD and 6PPD-quinone.

The results of the study are discussed in this presentation.

**2.07.P-We009 Effects of Low Dose Bifenthrin Exposure on Culex quinquefasciatus Pyrethroid Resistance**
***Nathan Darlucio Sy** and Jay Gan, University of California, Riverside*
The increased detection of pyrethroid insecticides in urban runoff has coincided with an increase in pyrethroid resistance in Culex quinquefasciatus mosquitoes in California. In addition to toxicity to non-target organisms, stormwater pyrethroid contamination may result in exposure to aquatic mosquito larvae, which frequently breed in storm drains, thus selecting for resistance. While bifenthrin is one of the most prevalent pyrethroids in stormwater, previous selection pressure research has focused on higher doses of other pyrethroids, such as permethrin and deltamethrin. To determine risks from this exposure scenario, the potential for bifenthrin to select for resistance in mosquitoes was examined. Cx. quinquefasciatus larvae were exposed to bifenthrin in various treatments, after which bioassays were conducted on adult mosquitoes to determine genotype, LD50, and other characteristics of resistance. Understanding low dose effects will help bridge the gap between previously-established toxicology and environmentally relevant concentrations.

**2.07.P-We012 Acute Toxicity of a Tire Rubber-Derived Chemicals, 6PPD-quinone and HMMM, to Various Life Stages of Atlantic Salmon (Salmo salar)**
***Danielle A. Philibert**[1], Michael Pirrung[2] and Benjamin Patrick de Jourdan[1], (1) Huntsman Marine Science*

*Center, Canada, (2) University of California*
Owing to numerous stressors salmon returns have been declining. One of the stressors that has been implicated in causing adult Coho salmon (*Oncorhynchus kisutch*) mortality is stormwater runoff from urban areas. Stormwater runoff is a complex environmental matrix, and recent ground breaking work has identified the causative agent within that mixture as *N*-(1,3-dimethylbutyl)-*N*'-phenyl-p-phenylenediamine-quinone (6PPD-quinone), which is formed by the environmental oxidation of a widely used tire anti-ozonate (6-PPD). In the wake of this discovery, 6PPD-quinone has been measured in various freshwater waterways around urban centers, along with other tire wear leachate compounds like hexamethoxymethylmelamine (HMMM). The toxicity of 6PPD-quinone has been assessed in other species, with results confirming a wide range of sensitivities to this compound, while little is known about the potential toxicity of HMMM. In this study we determined the toxicity of 6PPD-quinone and HMMM to Atlantic salmon (*Salmo salar*) fry (0.14 – 0.21 g), pre-smolt (66.3 – 219.4 g) and post-smolt (254.6 – 851.4 g) juveniles. Using synthesized 6PPD-quinone and commercially purchased HMMM, we conducted 24hr exposures with the freshwater (fry and pre-smolt) and seawater (post-smolt) life-stages of Atlantic salmon. Water samples were taken at 0, 3, 6, and 24hrs of exposure to measure the concentration of 6PPD-quinone and HMMM in the water using GC/MS. At concentrations up to 1.2 mg/L, HMMM caused no effects in any of the life stages of Atlantic salmon included in this study. 6PPD-quinone was most toxic to the fry with an LC50 of 19.7 µg/L, and no lethality was observed concentrations tested in our pre-and post-smolt salmonids. Following exposure, blood samples were collected from the pre- and post-smolts and hematocrit, glucose, lactate, red blood cell size and concentration, were determined, with only hematocrit in the pre-smolt showing an effect of exposure to 6PPD-quinone. This study highlights the importance of testing the toxicity of 6PPD-quinone on a range of different life stages, and the data generated will be used to inform management practices and provide insight into potential risks in watersheds across Atlantic Canada.

**2.07.P-We013 Mitigation Strategies for Tire Road Wear Particles at the Source and After Release into the Environment**

***Kelvin Gregory**[1], Ilka Gehrke[2], Jan Bloemer[2], Ralf Bertling[2], Hans-Joachim Unrau[3], Stefan Schläfle[3] and Melisa Öz[2], (1) Carnegie Mellon University, (2) Fraunhofer UMSICHT, Germany, (3) Karlsruhe Institute of Technology, Germany*
Tire road wear particles (TRWP) are introduced into the environment through abrasion of car tires by the road surface but also includes abraded road surface material. These particles vary in size, contain a variety of chemicals, and can emerge in stormwater from roadway runoff. As a result, concern about the fate and potential toxicity of these emerging contaminants has guided efforts to understand best practices that reduce the potential for adverse environmental impacts of TRWP. The presentation will describe the current understanding about effective strategies to mitigate TRWP. We will review the state-of-the-art systems for natural attenuation of the chemicals as well as engineered systems interception of the microplastics and potential for reduced toxicity of soluble components.

**2.07.P-We014 Water Quality Analysis for Increased Endocrine Disrupting Chemicals (EDCs) Detection Accuracy and EDC Relationship to Mosquitofish Masculinization**

***Jorge Luis Garcia Acosta**, Daniel H. Paull and Nora Demers, Florida Gulf Coast University*
Endocrine disrupting chemicals (EDCs) alter hormone responses leading to masculinization or feminization effects in organisms. EDCs are prolific and present in pesticides, plastics, personal care products, and fertilizers. Mosquitofish, *G. holbrooki*, serve as an important model organism for EDC research because they demonstrate physiological signs of masculinization when examining female anal fins. A common symptom of masculinization in female *G. holbrooki* is elongated anal fins similar to males. Typical females have a ratio of 1:1 when comparing anal fin rays 4 and 6, so if the ratio this benchmark indicates potential EDC presence in their environment. We hypothesized that there would be differences in EDC presence in water and

masculinization of female gambusia in different surface waters including stormwater runoff, reuse water used for irrigation, and communities using septic tanks.

Current methods for identifying particular EDCs using liquid chromatography-mass-mass spectrometry (LC-MS) yield varying results; thus, to increase detection specificity, new strategies are required. More sophisticated LC-MS equipment has led to more compound identity and concentration specificity. Yet the issue of varying results from one sample continues. Therefore, new integrated approaches coupling in situ and in silico methods are needed to ensure chemical identification and concentration accuracy.

This water quality analysis research will utilize LC-MS, single quadrupole to improve the detection and quantification of known EDCs from Lee County, Florida water samples from which *G. holbrooki* specimens have been collected and examined. Knowing the accurate EDC content present in the water and having measured abnormal anal fin development will help us better understand the relationship between the EDCs and their masculinization effect on the gambusia. We will report our findings. Documenting and reporting differences in surface water quality is the first step we can take to better understand what wastewater treatments need to be improved.

**2.07.P-We015 Analysis of tire-related chemicals in fish using liquid-chromatography coupled with tandem mass spectrometry**

***Denis da Silva****, Jonelle Gates and Irvin Schultz, National Oceanic and Atmospheric Administration (NOAA) Fisheries Northwest Fisheries Science Center*

6PPD [*N*-(1,3-dimethylbutyl)-*N'*-phenyl-*para*-phenylenediamine] is a chemical used during car tire manufacturing process to protect the tire rubber, which makes this chemical widely present in urban stormwater runoff. Recently, after a comprehensive investigation of stormwater and its toxicity, it was determined that a quinone transformation product of the 6PPD was one of the key compounds responsible for the pre-spawn mortality of coho salmon (*Oncorhynchus kisutch)*. This recent finding triggered a number of studies worldwide, developing analytical methods for measuring 6PPD and its quinone derivative 6PPD-Q, as well as determining the toxicity of 6PPD-Q in different fish species. However, most of the current methods of analysis are focused on measuring 6PPD-Q in water samples only. We developed a method for analysis of fish plasma and bile for four different tire-related chemicals (TRC) commonly found in stormwater runoff: 6PPD-quinone (6PPD-Q), N,N'-diphenylguanidine (DPG), N,N'-dicyclohexylmethylamine (DCA), and hexamethoxymethylmelamine (HMMM). Our analytical method includes a solid-phase extraction approach and final analysis by liquid-chromatography coupled with tandem mass spectrometry (LC-MS/MS) technique. This method proved to be fast, relatively inexpensive and sensitive, with a limit of quantitation around 10-100 pg/mL of sample. We intend to field validate this methodology using samples collected from urbanized and non-urbanized areas of Puget Sound, WA, while also determining the baseline levels of exposure to these TRCs in this region. Our analytical method aids assessments of fish exposures to these toxic compounds and their bioavailability, but also can be an important tool to help understand the distribution and toxic mechanisms of these chemicals in fish during lab-controlled exposure studies.

**2.07.P-We016 Controlling Saturation to Improve PFAS Removal in Biochar-Amended Stormwater Bioretention Systems**

***Kathleen M. Hawkins**[1], J. Conrad Pritchard[2], Yeo-Myoung Cho[2], Scott Struck[3] and Christopher Higgins[1], (1) Colorado School of Mines, (2) Stanford University, (3) Geosyntec Consultants, Inc.*

Stormwater is an important vector for per- and polyfluoroalkyl substances (PFAS) into ecosystems. Black carbon-amended bioretention systems are a popular strategy for contaminant removal in stormwater. PFAS preferentially accumulate at the air-water interface, but retention time requirements typically cause saturation to occur in bioretention systems, effectively removing the air-water interface. The goal of this study was to assess the effect of saturation on removal of PFASs, metals, and trace organic chemicals in simulated stormwater bioretention systems. A field-aged mixture of sand, zeolite and biochar was packed into columns which were operated with hydraulic controls to remain unsaturated or saturated during the duration of the experiment. Appropriately sized storm events were simulated using synthetic stormwater fortified with aqueous film forming foam-derived PFASs, TOrCs and metals to mimic real-world conditions. Unsaturated conditions outperformed saturated conditions for removal of all PFAS analyzed. The results have implications for bioretention system design for PFAS removal in contaminated catchment areas.

## 2.07.V Fate and Effects of Chemicals from Stormwater Runoff

### 2.07.V-01 Assessing the Health Effects of 6PPD-quinone on Newly Feeding Juvenile Chinook and Coho Salmon

***Bonnie Lo*[1,2]**, *Vicki Marlatt[1], Sofya Reiger[2], Carys Galilee[2] and Tanya Brown[2], (1) Simon Fraser University, Canada, (2) Fisheries and Oceans Canada*

The tire associated contaminant 6PPD-quinone has been identified as a serious threat to the health of juvenile and adult Coho salmon. Recent studies have reported a wide range of sensitivity to 6PPD-quinone among several fish species (e.g. Rainbow trout, Brook trout, Arctic char, Zebra fish), with Coho being by far, the most sensitive. Chinook salmon are among the salmon species whose sensitivity to 6PPD-quinone has not been established. In Canada and the United States, many populations of Chinook have been classified as either endangered or threatened under relevant federal endangered species laws. Juveniles from many populations (including 19 in the Fraser River, British Columbia), migrate through urban-impacted waterways prior to entering the ocean, thus risking exposure to 6PPD-quinone during an early, sensitive life-stage. Current 6PPD-quinone toxicity testing on juvenile salmonids has focused on the 1+ year range, therefore a data gap exists for younger fish. In this study, newly feeding (approximate 3 weeks) juvenile Chinook and Coho were exposed to 5 concentrations of 6PPD-quinone for 24 hours under static conditions. 6PPD-quinone in exposure water was measured at test initiation and termination. Mortality was assessed and LC50 values were calculated for each species. Results indicate that newly feeding juvenile Coho are more sensitive to 6PPD-quinone than 1+ year old fish. Also, the exposures demonstrated that juvenile Chinook, are an order of magnitude less sensitive to 6PPD-quinone than Coho. Tissue samples from these exposures will be analyzed using a variety of molecular and histopathological techniques. These analyses will assist in a deeper exploration of the sublethal changes occurring in exposed fish and may shed light on the potential mechanism of action of 6PPD-quinone and potential drivers of varying species sensitivity. Results from this research will be used to inform the risk of this prevalent urban contaminant to juvenile Chinook and Coho and aid in the conservation, recovery, and management efforts of these much-valued species.

## 2.08 Freshwater Salinization: Causes, Effects and Working Towards Solutions

### 2.08.T-01 The Salinization of Coastal Rivers from Sea Level Rise: Implications for Freshwater Mussels

***W. Gregory Cope*[1]**, *Joseph K. McIver II[1] and Ryan Boyles[2], (1) North Carolina State University, (2) U.S. Geological Survey*

Freshwater mussels inhabiting coastal rivers can be negatively impacted by water salinization. Rising sea levels, storm surges, and drought are known to alter salinity concentrations in these systems. Sea salt is largely made up of sodium ($Na^+$) and chloride ($Cl^-$) ions, forming NaCl, a known toxicant to freshwater mussels. However, sea salt contains other cations and anions, such as potassium, sulfate, and calcium. The collective impact of these ions on freshwater mussel fitness is not clearly understood. Therefore, we conducted acute toxicity tests on early life stages (glochidia and juvenile) of 3 freshwater mussel species that inhabit Atlantic Slope drainages (*Leptodea ochracea* (from a riverine habitat and a lacustrine habitat), *Ligumia nasuta*, and *Utterbackiana implicata*). Glochidia (larvae) and juveniles of each species were exposed to 7 concentrations of Instant Ocean® Sea Salt, a synthetic sea salt. These concentrations were 0, 1, 2, 8.5, 12.5, 17, and 34 parts per thousand (ppt). Glochidia underwent a 48-hour acute toxicity test, where viability was assessed at hour 24 and 48. Juveniles

underwent a 96-hour acute toxicity test, where viability was assessed at hour 48 and 96. We calculated the median lethal concentration (LC50) for each of the 8 acute toxicity tests and found that glochidia were more sensitive than juveniles to Instant Ocean® Sea Salt. LC50s for glochidia at hour 24 ranged from 0.45 to 3.95 ppt, with the most sensitive freshwater mussel being the *Leptodea ochracea* from the lacustrine habitat, exhibiting a LC50 of 0.45 ppt (95% C.I. = 0.39-0.51). Juvenile freshwater mussels exhibited 96-hour LC50s ranging from 5.04 to 7.90 ppt. Our results show that acute exposure to sea salt negatively impacts freshwater mussel viability, specifically glochidia. This information can be used to enhance freshwater mussel conservation strategies in regions that are impacted by seawater intrusion and inundation.

**2.08.T-02 The Influence of Water Hardness on the Salt-Sensitivity of Early Life Stage Freshwater Mussels.**

***Patricia Gillis***[1]*, Karen L. Lemon*[2]*, C. James Bennett*[1]*, Joseph Salerno*[1] *and Ryan S. Prosser*[2]*, (1) Environment and Climate Change Canada, (2) University of Guelph, Canada*

This study investigated how water hardness affects salt toxicity in two freshwater mussel species, *Lampsilis fasciola* and *Lampsilis siliquoidea*. Using standard toxicity testing methods the acute (24 and 48-hr) toxicity of sodium chloride to glochidia (i.e. freshwater mussel larvae) was examined in reconstituted waters of varying hardness. Reconstituted waters were created according to the US EPA (2002) recipe where varying prescribed amounts of $NaHCO_3$, $CaSO_4 \cdot 2H_2O$, $MgSO_4$, and KCl are added to distilled water. With the exception that the amount of potassium chloride added to create the hard and very hard waters was reduced from the indicated amounts (hard, 8 mg/L; very hard, 16 mg/L) to the moderately-hard water amount (i.e., 4 mg/L) as potassium was suspected to be responsible for reduced control viability in the harder waters. Hardness of the seven dilution waters employed ranged from 7 mg/L $CaCO_3$ (very soft water diluted 50:50 with distilled water) to 290 mg/L $CaCO_3$ (very hard water). Chloride concentrations in the exposures ranged from 5 to 5000 mg $Cl^-$/L depending on water hardness. Glochidia viability (ability to close valves) was used as a surrogate for survival to derive effect concentrations ($EC_{50}$). In both mussel species, increasing water hardness resulted in a sharp reduction in salt sensitivity up to a point (~150-200 mg $CaCO_3$/L, depending upon species) after which no further protection (i.e. increase in $EC_{50}$) was observed. The 48-hr $EC_{50s}$ for *L. fasciola* ranged from 7 mg $Cl^-$/L in very soft water (7 mg $CaCO_3$/L) to 390 mg $Cl^-$/L in hard water (180-190 mg $CaCO_3$/L). The 48-hr $EC_{50s}$ for *L. siliquoidea* ranged from 14 mg $Cl^-$/L in very soft water to 222 mg $Cl^-$/L in hard water. These data demonstrate that increased water hardness can provide some protection against salt toxicity to early life stage freshwater mussels.

**2.08.T-03 Interactions of Major Ions and Dissolved Organic Carbon on the Electrical Responses of the Fish Gill: A Study Using Model Compounds**

***Carolyn Morris****, Colin J. Brauner and Christopher M. Wood, University of British Columbia, Canada*

Exposure to elevated major ion levels disturbs ionoregulation in freshwater organisms. The MIT (Multi-Ion Toxicity) Model predicts the resulting salt toxicity as a function of the disturbance of the transepithelial potential (TEP, the electrical gradient across the gills between the fish and the external water). Dissolved organic carbon (DOC) has been found to be protective against ionoregulatory disturbances in freshwater fish, particularly at low environmental pH, including mitigating the often fatal increase in passive loss of major ions ($Na^+$, $Cl^-$) across the gills and lowering the TEP. Using the freshwater rainbow trout as our test organism, we investigated whether DOC and exposure to elevated major ions interact in the TEP responses. The complexity and heterogeneity of DOCs make them particularly difficult to study in the context of mechanistic investigations. Therefore, model compounds of known chemical structure were used, chosen based on the criteria that they structurally resemble and/or functionally behave like certain chemical moieties of typical humic substances (humic and fulvic acids) which are major components of DOC. Naturally sourced DOCs cause a decrease in TEP (i.e. more negative inside the animal) and our results show that some model compounds, including sodium dodecyl sulfate, tannic acid and Aldrich humic acid, show this same TEP response in a concentration dependent manner. However other chosen model compounds result in no change in

TEP or the opposite TEP response to natural DOCs. Our experiments show that increased concentrations of ambient major ions ($Na^{+}$ and $Cl^{-}$ from NaCl, $Ca^{2+}$ and $Cl^{-}$ from $CaCl_2$) cause increases in TEP in a concentration-dependent manner, and these changes may be altered in the presence of DOC model compounds. Possible interactive effects of pH are under investigation. Given that DOC is already recognized as an important protective agent against aquatic toxicity (in particular metals and low water pH) and has effects on ionoregulation comparable in magnitude to those of alkalinity, hardness, and salinity, we propose that DOC is a water quality parameter that should be considered for ecological understanding and risk assessments of freshwater salinization, such as those employing the MIT model (NSERC Discovery).

**2.08.T-04 Impacts of Salinity on Pesticide Toxicity in Fish**

***Daniel Schlenk***, *University of California, Riverside*

Sea level rise in estuarine locations where agricultural activities occur may lead to co-exposures of aquatic organisms to high salinity and pesticides. Over the past 30 years, numerous studies have been conducted showing that the impacts of salinity and pesticide co-exposures are species-specific with organisms having euryhaline life histories showing differential sensitivities to pesticides than those with stenohaline life histories. Many of the impacts have been associated with enhanced biotransformation to active metabolites that either target neurological targets (organophosphate/carbamate insecticides; chlorpyrifos/aldicarb) or endocrine targets (pyrethroids) causing potential reproductive or behavioral effects. Others have shown interactions with osmoregulatory targets through endocrine responses. This presentation will provide an overview of impacts caused by salinity and pesticide co-exposures and demonstrate the use of toxicity and adverse outcome pathway paradigms to identify key targets that can be used in weight of evidence components of ecological risk assessments.

**2.08.T-05 The Impact of Road Salt on Pacific Salmon Success**

***Christopher M. Wood***[1]*, Nikki Kroetsch*[2]*, Patricia Schulte*[1]*, Alan James*[3]*, Colin J. Brauner*[1]*, Anayansi Cohen-Fernandez*[4]*, Vicki Marlatt*[5] *and Christopher J. Kennedy*[5]*, (1) University of British Columbia, Canada, (2) Fisheries and Oceans Canada, Canada, (3) Stoney Creek Environment Committee, Canada, (4) British Columbia Institute of Technology, Canada, (5) Simon Fraser University, Canada*

Pacific salmon populations are in precipitous decline, and degradation of spawning habitat by contaminants has been identified as a significant cause. Road salt use in Canada is increasing at about 2.5% per year, and is very poorly regulated. Salt toxicity both to salmon early life stages and their food supply of benthic invertebrates may be a significant factor, especially in the very soft waters of the Pacific West Coast. The urbanized area of the Vancouver Lower Mainland contains many salmon spawning streams, and we have recently measured winter salt pulses that exceed water quality guidelines by 7- to 28-fold. We will give an overview of a recently started project that addresses this issue, both scientifically and socio-politically. The project is powered by Citizen Science - 13 local Streamkeeper organizations are donating extensive stream-monitoring effort (to establish the extent of the problem) and outreach activities (to increase public awareness). Additionally, network co-ordination and data management expertise is provided by Fisheries and Oceans Canada, and laboratory and field research by three Vancouver area Universities. The goals are: (1) establish a network of continuous year-round conductivity loggers to monitor salt pulses and chronic background levels in 30+ local spawning streams; (2) monitor the abundance of fish and benthic invertebrates in both impacted and non-impacted streams; (3) perform experimental laboratory studies on coho and chum salmon hatching, development, and physiology of the effects of winter/spring salt pulses of different types and temperatures, as well as chronically elevated salt levels. In-stream development assays will also be performed. The goal is to detect possible pathological effects and to understand their mechanisms; (4) perform parallel experimental studies on key benthic invertebrates to understand impacts on the forage base; and (5) mount a public outreach program to educate the general public, local governments, and regulatory authorities on the extent of the road salt pollution problem and its potential impacts on salmon success (NSERC Alliance Program).

**2.08.T-06 Addressing Salinization in Urban Streams Through Site-Specific Thresholds for Total Dissolved Solids Developed Using Toxicity Tests on Standard and Field-collected Species**

***Robert N. Brent***[1], *Jared Kunkel*[1], *Zachary Tomek*[1], *Dalton Buchardt*[1], *Peter De Lisle*[2] *and Sarah Sivers*[3], *(1) James Madison University, (2) Coastal Bioanalysts, (3) Virginia Department of Environmental Quality*

The increasing salinization of freshwater streams from anthropogenic land uses and activities is a growing global environmental problem. Increases in individual ions (such as sodium or chloride) and combined measures (such as total dissolved solids) threaten drinking water supplies, agricultural and economic interests, and the ecological health of freshwater streams. Because the toxicity of high ionic strength waters depends on the specific ion composition and background geology, few water quality standards exist to protect freshwater streams from salinization. In this study, we used a novel approach to develop site-specific and ecologically-relevant total dissolved solids (TDS) thresholds for the protection of aquatic life in Sand Branch, Loudoun County, Virginia, USA. Sand Branch is a small urban/suburban stream in the northern Virginia suburbs of the Washington D.C. metropolitan area. The stream has experienced salinization from multiple point and nonpoint sources including discharges from a hard rock quarry, concrete production facilities, and a composting facility, as well as nonpoint source runoff from winter deicing on roadways and commercial areas. Summertime baseline conductivity in Sand Branch is near 1000 µS/cm and wintertime peaks reach 3400 µS/cm. As a result, Sand Branch has a severely impaired benthic macroinvertebrate community and fails to support Virginia's aquatic life use. In addition, Sand Branch drains to the Occoquan Reservoir and contributes to the continuing salinization of this major drinking water source for northern Virginia. In this study, we used a combination of standardized toxicity test species and more ecologically relevant field-collected species to conduct toxicity tests on artificial samples prepared to match the ion composition of the impaired stream. From these tests, acute and chronic thresholds for TDS of 938 and 463 mg/L, respectively, were calculated. These thresholds will be used for Total Maximum Daily Load development in the watershed aimed at reducing TDS from point and nonpoint sources. The approach used in this project provides a potential model for establishing protective thresholds for other waterbodies impacted by salinization.

**2.08.T-07 Phytoremediation of Saline Soil Using Native Canadian Halophytes**

***Lauren Nawroth***[1], *Barb A. Zeeb*[2] *and Allison Rutter*[1], *(1) Queen's University, Canada, (2) Royal Military College, Canada*

Salt contamination is increasingly being identified as an environmental concern. Canada is one of the largest users of road salt worldwide; each year over 5 million tonnes of salt are distributed on roads to melt snow and ice. Inorganic chloride present in road salt has been found to pose a risk to freshwater ecosystems, terrestrial vegetation, and wildlife. The copious amounts of salt applied in winter have lasting effects in ecosystems located roadside and beyond, throughout the summer months. Other notable sources of salt contamination in the environment include oil and gas production, and the production of saline material from various industries. Traditional methods of remediation are often costly and labour intensive with negative impacts on the environment due to the generation of toxic by-products, accelerated soil erosion, and edaphic soil conditions. Phytoremediation utilizing halophytes (i.e. salt tolerant plants), is being investigated as a way of remediating salt-impacted roadsides. Previous greenhouse and small-scale *in situ* studies have shown significant reductions in soil salinity using halophytes. In this study, native Canadian halophytes are being evaluated for their abilities to remediate salt contaminated soils at two different field sites. One site is located adjacent to an international airport where roads are treated with salts throughout the winter months for safety reasons, while the second site is an industrial location previously storing batteries. The contrasts of the two field sites will allow for a comprehensive analysis of halophytic abilities in large scale applications. This paper will report on the 2022 results from remediation efforts at both field sites, as well as on concurrent greenhouse trials being carried out at Queen's University.

**2.08.T-08 Discussion: Freshwater Salinization: Causes, Effects and Working Towards Solutions**

***Patricia Gillis***, *Environment and Climate Change Canada*

Salinization of freshwater is a growing global concern. The information presented in this session will contribute to our understanding of the risk salinization poses to sensitive biota and can inform mitigation and management efforts. Session presenters (platform and posters) and chairs will be assembled for a panel discussion to consider salt-vulnerable ecosystems and the various approaches to best protect them. The panelists hold expertise on characterising salinity and salt toxicity, developing predictive models, and establishing protective thresholds for salinization-impacted waterbodies. In addition they can speak to remediating salt-impacted sites and generating total dissolved solids and chloride toxicity data to inform water quality regulations, among other areas.

**2.08.P Freshwater Salinization: Causes, Effects and Working Towards Solutions**

**2.08.P-Tu050 Evaluating the Ability of Eisena fetida to Recover Following Road Salt Exposure**
***Rebecca E. Yates*** *and Amanda D. Harwood, Alma College*
Millions of tons of road salts are applied yearly in the United States. Runoff of road salts into soils and waterways causes adverse effects to both terrestrial and aquatic organisms. The toxicity of road salts has been frequently studied in the laboratory using macroinvertebrates such as *Eisenia fetida*. While the toxicity of road salts is commonly studied and understood, fewer studies have been conducted on the lasting effects of road salts on these organisms following removal from exposure. Therefore, the objective of the current study was to evaluate the ability of juvenile *E. fetida* to recover following road salt exposure. In this study, toxicity and recovery were evaluated for two different salts, sodium chloride and beet deicer. We conducted 28-d exposures using a range of salt concentrations with a 28-d recovery period following. Mortality, change in weight, and development of clitellum were observed post-exposure period and post-recovery period at each concentration to determine recovery potential. The current study should provide insight to how well ecosystems can recover following exposure, such as in periods of low road salt exposure or following remediation projects, and how effective remedial efforts may be.

**2.08.P-Tu051 The Effect of Diet on the Sensitivity of Hyalella azteca to De-icing Agents**
***Greysen R. Tomlinson*** *and Amanda D. Harwood, Alma College*
The salting of roads results in contamination of local waterways, having various impacts on aquatic systems. The environmental factors that may influence this toxicity are less understood. Since the highest exposure to road salts may coincide with reduced food availability or quality during winter months, the role of diet in salt toxicity should be evaluated. The current study evaluates the effect of diet on the sensitivity of adult and juvenile *Hyalella azteca* to sodium chloride and beet deicer. Several commonly used *Hyalella* diets were tested including TetraMin, TetraMin and diatom combinations, and conditioned *Acer sacharum* leaves. A 48 h toxicity bioassay was conducted for juveniles and adults cultured on each diet. To further evaluate the effects of diet, these results were compared to animals with a 96 h starvation period prior to exposure. This study will help provide preliminary data on the role of diet in the toxicity of road salts.

**2.08.P-Tu052 Characterization of Surface Water Salinity in the Lake Winnipeg Watershed**
***Braedon William Humeniuk***[1]*, Pepe Rodriguez-Gil*[2] *and Mark L. Hanson*[1]*, (1) University of Manitoba, Canada, (2) IISD Experimental Lakes Area (IISD-ELA), Canada*
The concentrations of salts in freshwater systems have been increasing globally (from both natural and anthropogenic sources), making the ecological impacts of salinization a pressing concern. Chloride ions are highly mobile, and retention times are typically longer in lakes and wetlands; therefore, relatively low inputs may result in elevated concentrations over time. We are interested in characterizing the current state of salinity and salinization in the Lake Winnipeg watershed, as evaluated through the lens of a community-based monitoring program. Volunteers gathered samples over two field seasons (2020 and 2021), from 170 sites across Manitoba, northwestern Ontario, and North Dakota, with approximately 20 samples per site collected per year. The exposure concentrations will be compared with freshwater chloride toxicity data to assess the risk to

sensitive species. This information will be used in conjunction with land-use data and other geographically explicit datasets to determine the possible sources and drivers of salinity in the Lake Winnipeg watershed. During the 2020 and 2021 field seasons, multiple sites exceeded freshwater limits and can be classified as brackish water (>1500 µS/cm), as well as exceedances of the chronic Canadian Water Quality Guidelines for the Chloride Ion and the United States Environmental Protection Agency's National Aquatic Life Criteria for Chloride (>500 mg/L of chloride). Sites that experienced the greatest salinity levels were in the Red River Valley and near the city of Winnipeg, Manitoba, suggesting that anthropogenic factors may be driving salinity in these regions. Lake Winnipeg is among the largest freshwater lakes in the world, and with such an expansive drainage basin (spanning four Provinces and four States), there are numerous potential pathways for dissolved salts to enter Canadian surface waters. Therefore, high quality exposure data such as these are needed to inform risk assessors when making regulatory decisions regarding water protection in the country.

**2.08.P-Tu053 Connecting in-stream toxicity testing with community-level responses in an East Tennessee stream**

***Louise M. Stevenson****, Paul Matson, Natalie Griffiths, Christopher DeRolph, R. Trent Jett, Allison Fortner, Nikki Jones and Teresa Mathews, Oak Ridge National Laboratory*

Laboratory toxicity tests represent a standard method to assess the potential impairment of freshwaters by anthropogenic activities. While this standardized, lab-based approach provides some useful information on potential stressors to a system, it is unclear whether these data provide insight regarding community-level impacts of toxicity on aquatic organisms in the field. As part of Biological Monitoring and Abatement Program at Oak Ridge National Laboratory, we monitor ecological impacts, evaluate remediation effectiveness, and document ecological recovery in multiple streams on the Oak Ridge Reservation, a federally owned 33,476-acre site in east Tennessee managed by the US Department of Energy. Here we present data from in-stream toxicity testing, contaminant bioaccumulation assessments, and macroinvertebrate and fish community surveys conducted from the early 1990s to 2022 at various sites along Bear Creek, a 2$^{nd}$ to 4$^{th}$ order perennial stream, downstream from the Y-12 National Security Complex. This rich data set offers us a rare opportunity to test the strength of in-stream toxicity testing to predict ecological effects on in-stream communities across decades. The toxicity testing data set consists of biannual three-brood *Ceriodaphnia dubia* tests from 2009 to 2022, and significant impacts of the stream water on *C. dubia* reproduction have been observed at certain sites through time. Analysis of water quality data in conjunction with these responses has revealed that water quality parameters, specifically conductivity, have a strong correlation with observed sublethal toxicity to the *C. dubia.* Using precipitation, flow, and other environmental data sets, we investigated the drivers connecting conductivity and toxicity. Further, we attempted to connect periods of sublethal toxicity, based on the toxicity test results, to observed changes in invertebrate and fish community metrics over time. These extensive, multi-decadal data sets allow us to investigate both the potential for in-stream toxicity test results to predict in-stream changes in aquatic insect and fish communities and potentially identify the abiotic drivers of these responses.

**2.08.V Freshwater Salinization: Causes, Effects and Working Towards Solutions**

**2.08.V-01 Transepithelial Potential Responses of Fish to Major Salts: The Influence of Water Hardness**

***Beverly Po*** *and Christopher M. Wood, University of British Columbia, Canada*

The transepithelial potential (TEP) of freshwater organisms is currently being evaluated for its relationship to the toxicity of major salts, in support of the development of the Multi-Ion Toxicity (MIT) Model. While previous data showed potential influence of different water hardness, as calcium level, on TEP, there is a lack of direct comparisons. With the use of the fish model Fathead Minnow (*Pimephales promelas*), we investigated how water calcium level might affect the TEP and transport of major salts. Acute major salt serial exposures ($NaCl$, $Na_2SO_4$, $NaHCO_3$, $KCl$, $K_2SO_4$, $KHCO_3$, $CaCl_2$, $CaSO_4$, $MgCl_2$, $MgSO_4$) under different water hardness, and exposure after prolonged acclimation to different calcium level were involved. We found a lowered baseline TEP in calcium-elevated water, resulting in higher TEP change in salt series. However, the general trend of TEP change along salt concentration remained consistent, with TEP reaching maximum at concentration close to the LC50s. This trend was reserved with different strains of fish and in different background water hardness.

**2.08.V-02 Assessing the Impacts of Chloride on Aquatic Life in a Headwater Tributary**

*Daniel Guth, Ryan Holem and* ***Robert Gensemer****, GEI Consultants, Inc.*

Chloride can have significant harmful effects on aquatic life, particularly freshwater mussels and other aquatic invertebrates. Sources of chloride to aquatic systems include but are not limited to runoff from road salt application, water softeners, and industrial discharges. Facilities such as wastewater treatment plants may be faced with rising treatment costs to comply with chloride limits in NPDES permits. Herein we present results from a project evaluating the extent to which aquatic life uses are being protected downstream of a municipal effluent that is exceeding regulatory standards for chloride. Biological and habitat surveys were completed downstream of the effluent and in nearby comparison/reference streams. In addition to the biological surveys, surface water samples were collected to conduct aquatic toxicity testing and to run toxicological models to better understand the effects of chloride on this headwater system. Despite predicted and measured toxicity in the waters downstream of the outfall, our results support that habitat characteristics (e.g., substrate heterogeneity, instream cover, etc.) are more closely associated with aquatic community structure than elevated chloride concentrations. In addition, elevated chloride may not be a primary factor to attaining aquatic life uses downstream of the facility.

**2.09 Latest Advances in Metal Bioavailability and Toxicity to Aquatic Organisms**

**2.09.T-01 Introduction: Latest advances in metal bioavailability and toxicity to aquatic organisms**

***Severine Le Faucheur***[1] *and Anne Cremazy*[2]*, (1) Université de Pau et des Pays de l'Adour, France, (2) University of New Brunswick, Canada*

**2.09.T-02 Low Levels of Metals in Mixture with Chelating Pesticides Exert Renal Toxicity in Zebrafish, Early-life Through Adulthood, and How Heat Waves Interact with This Toxicity**

***Ilaria Merutka***[1]*, Remy Babich*[2]*, Kelsie Dougherty*[1]*, Melissa Chernick*[1]*, Kasun Gunawardena*[1]*, Iain Drummond*[3]*, Lee Ferguson*[1]*, P. Mangala De Silva*[4] *and Nishad Jayasundara*[1]*, (1) Duke University, (2) University of Maine, (3) Mount Desert Island Biological Laboratory, (4)University of Ruhuna, Sri Lanka*

Numerous pesticides are known chelators of metals which increases the lifespan and availability of these chemicals in water and may modulate adverse health effects. Here, we examine the role of heavy metals and chelating pesticide glyphosate in accelerated kidney damage, even at levels below regulatory limits. Our study is motivated by the association of these contaminants with a high prevalence of chronic kidney disease of unknown etiology in tropical farming communities. These regions have populations and aquatic ecosystems exposed to agrochemicals and increasingly frequent, intense heat waves due to climate change. The primary questions of this research are 1) how environmental levels of metals (As, Cd, Pb, V) and glyphosate (3-15 ppb) induce developmental and renal toxicity and 2) how heat waves interact with health effects of these chemicals. We expose zebrafish (*Danio rerio*) to a mixture of metals and glyphosate with intermittent raised temperatures, from embryo continuing into adulthood, to investigate systematic mitochondrial, developmental, and renal tissue damage that may accumulate.

We show exposure to permutations of metals and glyphosate in mixture produce differential mitochondrial respiration and gene expression patterns in zebrafish renal development. Further, RNASeq analysis reveals distinct patterns of gene changes between the metals mix, glyphosate alone, and metals mix with glyphosate. The metals mix heavily targets DNA modification in what may be an adaptive response, while metals with glyphosate perturb fibrotic and lysosomal processing, both disease phenotypes in mammalian kidney. Early-life exposure to metals and glyphosate lead to rapid changes in zebrafish activity level and mitochondrial respiration in a dose-dependent manner. Further dose response are being conducted to explore thresholding of these early effects. Renal histology is underway to characterize subcellular and morphological damage from organ development through adulthood. Results suggest that intermittent mild heat mitigates early mitochondrial effects but may exacerbate chemical-driven renal dysmorphia and vacuolization in later organogenesis. In total, our data show systematic and renal toxicity from exposure to low levels of metals and glyphosate, mixture effects from permutation exposures, and that mild heat interacts with these effects, indicating the need to integrate effects of metals, chelators, and climate stressors in aquatic organisms and human health.

**2.09.T-03 Assessing the Vulnerability of Northern Salmonid Species to Combined Trace Metal Contamination and High Temperature Stressors**

***Mackenzie Anne Clifford Martyniuk**[1], Anthony Fontaine[2], Camille Garnier[1] and Patrice Couture[1], (1) Institut National de la Recherche Scientifique (INRS), Canada, (2) Université de Pau et des Pays de l'Adour, France*

When subjected to heat stress, fish are less resistant to contaminants. As a result, it is anticipated that climate change will have negative effects on fish health and condition in areas in proximate to mining operations. However, previous research examining these combined stressors has been restricted to species endemic to more temperate regions, despite more northerly distributed fish being of significant cultural and economic importance to local communities. Therefore, using both field and laboratory studies, this research aims to not only increase the availability of ecotoxicological knowledge detailing trace metal uptake and handling in Northern salmonid fish, but also establish critical thresholds of metal contamination in association with temperatures anticipated under climate change models. The study's field component included environmental and salmonid fish tissue collections from mining impacted zones in Northern Québec, Canada during the summers of 2019 and 2021 to detail current gradients of metal contamination, as well as ecotoxicological impacts on fish health and condition. Field collected data were then used to inform laboratory studies that focused on determining critical thresholds of metal contamination. This was accomplished through the assessment of health and condition indicators, as well as metabolic performance in Arctic charr to combined heat and metal stressors via cadmium exposure experiments at current (6°C) and anticipated (16°C) summer temperatures for this species. Preliminary results indicate that temperature stressors have a greater negative effect on indicators of fish growth than trace metal stressors alone. Additionally, combined trace metal and high temperature stressors appear to promote increased elemental uptake in Arctic charr, as significantly elevated cadmium concentrations were observed in all assessed tissues. Evaluated together, results suggest that climate change may pose an increased risk to the health of salmonids situated near mining operations. Finally, all collected field and laboratory data will be modeled in association with surface warming predictions to determine critical scenarios for salmonids in Northern Québec in an effort to ensure the continued preservation and informed management of these culturally and economically important species.

**2.09.T-04 Effects of Nickel Exposure on Aquatic Invertebrates: An Arctic Perspective**

***Connor B. Stewart**[1], Patrick Gauthier[1], Hannah Lowes[1], Robert Griffin[1], Emily Rogevich Garman[2], Anne Cremazy[3] and Tamzin A Blewett[1], (1) University of Alberta, Canada, (2) NiPERA, Inc., (3) University of New Brunswick, Canada*

Nickel (Ni) is an essential trace metal of economic interest, however, increases in global production have subsequently led to industrially contaminated areas. In excess, Ni is toxic, but the toxicity of Ni, and other metals is dictated by the concentration of free metal ion, which is influenced by organic and inorganic water constituents that alter bioavailability. Risk associated with Ni in aquatic environments has been relatively well defined in various temperate and tropical systems, but there is limited information on the risks Ni poses in the Arctic. Global warming is making the Arctic more accessible for natural resource extraction, increasing potential contamination. Understanding the threat posed by Ni exposure to the unique flora and fauna of the Arctic is critical. We aimed to understand the sensitivity of Arctic echinoderms to Ni, comparing them to native temperate species from the west coast of Canada. We conducted 96-hour effect concentration (EC50) exposures to Ni in three sea urchin species: S*trongylocentrotus droebachiensis*, *Strongylocentrotus purpuratus*, and

*Mesocentrotus franciscanus*, with *S. droebachiensis* being a representative Arctic species. The Arctic *S. droebachiensis* was the most sensitive species to Ni with a nominal $EC_{50}$ of 21.5 µg/L (95% CI 19.4 - 23.6), compared to 255 µg/L (95% CI 216 - 305) and 371 µg/L (95% CI 309 - 457) in *S. purpuratus* and *M. franciscanus*, respectively. These results suggest the Arctic relevant *S. droebachiensis*, are potentially at a greater risk from Ni contamination than the more temperate *S. purpuratus* and *M. franciscanus*. To further our understanding of risks posed by Ni in Arctic systems, acute and chronic toxicity testing will be conducted on the freshwater cladoceran *Daphnia pulex*, which are relevant to Arctic freshwater systems and are known to be sensitive to Ni. This study will evaluate Ni toxicity in a variety of waters that mimic Arctic relevant parameters, including pH, temperature, and hardness. Incorporation of both lethal and sublethal endpoints will improve knowledge of the risks posed by Ni in Arctic environments. These results taken together will provide insight into the Ni sensitivity of animals relevant to Arctic and near-Arctic ecosystems, thus developing our understanding of the potential risks Ni in the Arctic. Funding provided by NiPERA and NSERC Discovery grants program to TBlewett.

**2.09.T-05 The Roles of Calcium and Salinity in Protecting against Physiological Symptoms of Waterborne Zinc Toxicity in the Euryhaline Killifish (Fundulus heteroclitus)**

*Vania Loro[1] and **Christopher M. Wood**[2], (1) Universidade Federal de Santa Maria, Brazil, (2) University of British Columbia, Canada*

In fresh water, environmental Ca ameliorates Zn toxicity because $Ca^{2+}$ and $Zn^{2+}$ compete for uptake at the gills. Zn toxicity is also lower in sea water, but it is unclear whether this is due to increased $Ca^{2+}$ concentration, and/or to the other ions present at higher salinity. Using the euryhaline killifish, we evaluated the relative roles of $Ca^{2+}$ (as $CaNO_3$) versus the other ions contributing to salinity in protecting against physiological symptoms of $Zn^{2+}$ toxicity. Killifish were exposed to a sublethal level of Zn (500 µg/L, as $ZnSO_4$) for 96h in either fresh water (0% salinity) at low (1 mmol/L) and high Ca (10 mmol/L) or 35 ppt sea water (100% salinity) at low (1 mmol/L) and high Ca (10 mmol/L). At 0% salinity, high Ca partly or completely protected against the following effects of Zn seen at low Ca: elevated plasma Zn, hypocalcaemia, inhibited unidirectional $Ca^{2+}$ influx, inhibited branchial $Na^+/K^+$ATPase and $Ca^{2+}$ATPase activities, and oxidative stress in gills, liver, intestine, and muscle. At 100% salinity, in the presence of 1 mmol/L (low Ca), Zn caused no disturbances in most of these same parameters, showing that the "non-Ca" components of sea water alone provided complete protection. However, for a few endpoints (inhibited intestinal $Ca^{2+}$ATPase activity, oxidative stress in gill and liver), high Ca (10 mmol/L) was needed to provide full protection against Zn in 100% salinity. There was no instance where the combination of 100% salinity and high Ca failed to provide complete protection against Zn-induced disturbances in sea water. (NSERC Canada Discovery, CNPq Brazil).

**2.09.T-06 Transcriptome Responses of Rainbow Trout and Chinook Salmon to Sub-Lethal Exposures to Zinc**

***Jay R. Reichman**[1], Jeff A. Steevens[2], Aïda Farag[2], David Harper[2], Nile E. Kemble[2], Russ Hockett[1], Drew R. Ekman[1], Hongzu Ren[1], Anna Fisher[1] and Mark Jankowski[1], (1) U.S. Environmental Protection Agency, (2) U.S. Geological Survey*

In the environment, fish are often exposed to cationic metals from mining activities, roadway runoff, and other sources. Data are needed on the influence of taxonomic relatedness regarding the molecular impacts from metal exposures among closely related taxa to help inform decisions on the potential use of surrogate species in toxicity risk assessments. Thus, we compared transcriptome changes in fin, gill, and liver of juvenile rainbow trout (*Oncorhynchus mykiss*) and Chinook salmon (*Oncorhynchus tshawytscha*) exposed to zinc (Zn) in control laboratory waters, and at 17%, or 50% of available 96-hour LC50 concentrations for 24 or 96 hours (N=6 per experimental group). Trimmed Illumina RNA-Seq reads from trout and salmon were aligned to GCF_002163495_1_Omyk_1.0_rna and GCF_018296145.1_Otsh_v2.0_rna templates respectively. Expression levels compared to controls were normalized with DESeq2 and differentially expressed genes (DEGs) were identified (i.e., ≥2-fold change, corrected $P \leq 0.05$ and signal ≥10). Enrichment of KEGG and Reactome pathways by DEGs was tested by Fisher's Exact Test (corrected FDR = 0.05). Rainbow trout had 1632 DEGs across all groups, while there were 10809 in Chinook salmon. For both species, the majority of DEGs came from gill tissue (58% and 96%, respectively). There were 88 significantly enriched pathways for trout and 211 for salmon with 21 shared between the species. For both species there were proportional increases of impacted pathways associated with higher Zn concentration and longer exposure duration. The top hierarchical categories for the shared pathways were *cell cycle, cellular responses to stimuli, environmental information processing, extracellular matrix organization, genetic information processing, hemostasis, immune system, metabolism, metabolism of RNA, organismal systems,* and *signal transduction*. The results suggest that a diverse core of salmonid-specific transcriptome responses to Zn exposure is present within the broader changes seen in each species. Investigations are underway to identify the extent to which these pathways are also affected by Zn in more distantly related fish including zebrafish (*Danio rerio*) and medaka (*Oryzias latipes*). In addition, we are checking for relationships between salmonid transcriptome changes and metabolomic shifts.

**2.09.T-07 Metabolomic Response of Biofilms Exposed to Cobalt in Short-Term Experiments**

*Simon Colas[1], Benjamin Marie[2], Mathieu Milhe-Poutingon[1], Patrick Baldoni-Andrey[3], Amiel Boullemant[4], Clementine Gelber[5], Claude Fortin[6] and* ***Severine Le Faucheur****[1], (1) Université de Pau et des Pays de l'Adour, France, (2) Muséum National dHistoire Naturelle, France, (3) TOTALENERGIES, France, (4) Rio Tinto Aluminium Pechiney, (5)TOTAL S.A., France, (6)Institut National de la Recherche Scientifique (INRS), Canada*

Metabolites are low molecular weight molecules produced by organisms upon cellular activities such as photosynthesis, homeostasis processes or detoxification. They can thus be representative of modifications of cellular metabolism induced by changes in ambient water quality of organisms, such as increase in metal concentrations. Up to now, most studies have focused on examining either metabolomic responses in one aquatic organism at a time or one metabolite in microorganism community, such as biofilms. In the present study, we aimed to evaluate modification of biofilm metabolome upon short-term exposure to cobalt to assess its potential use of early-warning biomarkers of Co contamination in freshwater.

To that end, mature biofilms were exposed for 7 days in outdoor microcosms (15 L) to increasing concentrations of Co (4 in total), from $10^{-7}$ mol.L$^{-1}$ (5.9 mg.L$^{-1}$) to $10^{-5}$ mol.L$^{-1}$ (589 mg.L$^{-1}$). The temperature, pH and dissolved metal concentrations were monitored every day in exposure media whereas concentrations of the major cations, anions and dissolved organic carbon were measured at the beginning of the experiment. Biofilm samples were collected after 1, 3 and 7 days of exposure and were examined for their Co bioaccumulation (total and intracellular), biomass, chlorophyll concentration, and metabolic content using an untargeted approach.

Very good correlations were observed between the logarithm of the dissolved Co concentration and the intracellular Co content at each exposure time ($R^2_{adj}$: 0.91, 0.95, 0.97 at days 1, 3 and 7, respectively). Biomass was negatively impacted at the two highest Co concentrations with a 35% decrease at $10^{-5}$ mol.L$^{-1}$ after 7 days of exposure. A decrease in chlorophyll content was also observed at each studied concentrations at the longer time of exposure. In total, 2117 metabolites were measured in biofilms, with 159 being annotated. Among those annotated, 39 were dysregulated by Co. A decrease in flavonoid synthesis was observed whereas an increase in lipid production or its precursors (phosphatidylethanolamine, sphingosine and lysodiacylglyceryltrimethyl-homoserines) was measured. Current analyses focused on drawing dose-response curves based on annotated and unannotated metabolites in order to asses Co effects on biofilms.

**2.09.T-08 Use of Microchemical Analysis of Gastropod Shells to Assess Metal Exposure in Rivers**

*Maëva Marimoutou[1], Gilles Bareille[2], Patrick Baldoni-Andrey[3], Amiel Boullemant[4], Cécile Courreges[2], Clementine Gelber[5], Sandra Mounicou[2], Helene Tabouret[2] and* ***Severine Le Faucheur****[2], (1) Institute of Analytical Sciences and Physico-Chemistry for Environment and Materials (IPREM), France, (2) University of Pau and Pays de l'Adour, France, (3) TOTALENERGIES, France, (4) Rio Tinto Aluminium Pechiney, (5)*

*TOTAL S.A., France*
Gastropod shells develop throughout their lives by integrating some of the chemical elements present in their environment, including metals. Although studies are scarce, relationships have been established between the distribution of metals in the whole shell and in the surrounding environment. The objective of this work is therefore to evaluate the use of shells as a biomonitoring tool for metal concentrations in freshwater.

To this end, the gastropods *Radix balthica* were collected at different sites influenced by the input of metals related to the discharge of a wastewater treatment plant (WWTP). The sites chosen were: 1) upstream of the WWTP, 2) in the mixture zone of the WWTP effluent with river water and 3) downstream of the WWTP. Microchemical analyses of metals in the shell layers were performed by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) while analyses of the molecular environment of metals were performed by time-of-flight secondary ion mass spectrometry (TOF-SIMS). Shells were also analyzed for their total metal content by inductively coupled plasma mass spectrometry (ICP-MS) after acid digestion. The temperature, pH and concentrations of cations, anions and dissolved organic matter were measured at each sampling site.

The LA-ICP-MS results showed that Sr, Ba and Mn were homogeneously distributed in the shells. Their highest concentrations, *i.e.,* 1083 µg/g for Sr, 126 µg/g for Ba and 168 µg/g for Mn, were found in shells collected at the upstream site. For Zn (85 µg/g), Ni (23 µg/g), Pb (17 µg/g) and Cu (10 µg/g), their maximum shell content was in gastropods collected in the effluent but those metals were mainly accumulated in a thin layer (15 µm) on the outer shell surface. That layer was determined to be enriched in polypeptides and phospholipids by TOF-SIMS. A relationship was found between metal concentrations in ambient water and those in shells when using their specific distribution in shells rather their total concentrations. These results suggest that gastropod shells could be interesting bioarchives for assessing metal exposure in freshwater environments.

## 2.09.P Latest Advances in Metal Bioavailability and Toxicity to Aquatic Organisms

### 2.09.P-Mo074 Effects of Taxa and Body Size on Mercury Contamination of Riparian Spiders: Implications for the Use of Spiders as Sentinels

*Andrew Charles Todd[1], Ray Drenner[1], Madeline Pratt Hannappel[2], Morgan Capone[1], Christopher Allender[1], Robert Peterson[1], Tyler Williams[1] and* ***Matthew M. Chumchal****[1], (1) Texas Christian University, (2) University of North Texas*
Riparian spiders are exposed to mercury (Hg) through the consumption of emergent aquatic insects and have been proposed as sentinels of aquatic Hg contamination. Although riparian spiders show promise as sentinels that are used to characterize bioavailable chemical contaminants in food webs, few studies have explored the biological and ecological factors that may influence Hg concentrations in the tissues of sentinel spiders. The objective of this study was to assess the effects of spider taxa and body size on Hg concentrations of four taxa of riparian spiders (*Larinioides* sp., *Tetragnatha* sp., *Rabidosa* sp., and *Pardosa* sp.) collected from two rivers with different levels of Hg contamination. Spiders were collected from the Clear Fork (previously found to have high Hg contamination) and West Fork (previously found to have low Hg contamination) of the Trinity River, Fort Worth, Texas, USA in May and June of 2021. Average concentrations of total Hg (THg) in all taxa of riparian spiders were significantly higher on the Clear Fork than the West Fork. Within each river, THg concentrations differed between spider taxa and were positively correlated with spider body size in 3 out of 4 spider taxa. These findings suggest that future studies must take these biological and ecological factors into account when using riparian spiders as sentinels.

### 2.09.P-Mo075 Effect of Rare Earth Elements Nd, Pr and Y, Individually and in Mixtures to Daphnia magna

***Celine Do****, Scott Smith and Jim McGeer, Wilfrid Laurier University, Canada*

Neodymium (Nd), Praseodymium (Pr), and Yttrium (Y) are three rare earth elements (REEs) that occur naturally together in a mineral ore called bastnaesite, which is the primary ore of Canada's first rare earth element (REE) mine at Nechalacho, NT. These elements are increasingly being used in modern technologies and as such, there is an increasing concern for the potential environmental risk associated with anthropogenic contamination. In addition to there being very little data available for individual REEs, there is also a lack of knowledge concerning mixtures. The objective of my study was to investigate the acute effects of Nd, Pr and Y as a single metal and as a ternary mixture to *Daphnia magna*. Standard 48-h single metal acute tests following Environment and Climate Change Canada methods were conducted with *D. magna* neonates in an artificial soft water medium with a hardness of 50 mg $CaCO_3$/L and a pH of 6.8 with no added bicarbonate to determine the $EC_{50}$ for each individual metal. For example, the 48-h $EC_{50}$ for Y was 0.54 mg/L (95% CI of 0.48-0.62 mg/L) and for Nd it was 0.32 mg/L (95% CI of 0.03 - 0.56 mg/L). Mixture exposures were designed using a toxic unit (TU) approach, based on converting the $EC_{50}$ concentrations to toxic units and applying a matrix isobologram approach. For example, in the case of acute responses to mixtures, TUs of 0.25, 0.5, and 0.75 were prepared to observe whether the responses would follow an additive interaction. This research is supported via a NSERC Strategic Grant with additional funding from Environment and Climate Change Canada and contributions from Avalon Rare Metals Inc.

**2.09.P-Mo076 Risks of Gadolinium in Urbanized Aquatic Environments, Is There a Concern?**
***Johann Memmel****, Andrea Lister, Scott Smith and Jim McGeer, Wilfrid Laurier University, Canada*
Gadolinium (Gd) is primarily used in contrast agents for magnetic resonance imaging (MRI), with additional applications in metallurgy, and neutron shielding, and other niche applications. Both Gd and Gd-based contrast agents (GBCAs, the form used for MRIs) have been detected downstream of wastewater effluent discharge and there is currently little research into the risks to aquatic ecosystems. This study investigates the potential environmental risks of Gd and GBCAs using the Grand River in southwestern Ontario as a model ecosystem because it contains a variety of land uses (urban, industrial, agricultural) as well as multiple MRI facilities that use GBCAs. Total and dissolved REE concentrations were measured along the Grand River and its tributaries in different seasons with a focus on potential sources of contamination such as wastewater treatment plants. Sampling results showed low but measurable levels of multiple REEs in the watershed, with Gd anomalies downstream of wastewater discharge points of up to 407% the concentrations upstream (0.171 µg/L at outflow & 0.042 µg/L upstream). The potential for adverse effects of inorganic Gd and GBCAs were assessed using standard toxicity test methods with *Daphnia magna* as well as tissue bioaccumulation studies with fish (rainbow trout and fathead minnows). Results show that calcium and dissolved organic matter reduce toxicity by reducing the bioavailability and uptake of dissolved Gd. Carbonates were also important and increased levels induced significant precipitation. Trout exposed to 15 mg Gd/L (nominal) for 24h, in a soft water with no added bicarbonate (pH 6.8, hardness 50 mg $CaCO_3$/L,), had gill burdens of 183 mg Gd/kg, compared to 9.8 mg/kg in media at the same total Gd concentration but with 1mM $HCO_3^-$ added. In the soft water dissolved concentrations of Gd were 94.5% of total Gd while with added bicarbonate it was only 1.4%. Chronic 21d reproduction tests with *D. magna*, in the same soft water media showed no significant effect on survival or reproduction in concentrations up to 200 µg/L inorganic Gd. This study will contribute towards understanding the risks associated with Gd and GBCAs. Research supported by a NSERC Strategic grant with funding from Environment and Climate Change Canada and additional contributions from Avalon Advanced Materials Inc.

**2.09.P-Mo077 Understanding Elemental Profiles of Elizabeth River, VA in Environmental Samples**
***Samantha Alexandra Murphy****, Nelson Rivera, Heileen Hsu-Kim, Richard Di Giulio, Joel Meyer and Nishad Jayasundara, Duke University*
**Background:** The Elizabeth River in Virginia has been studied for organic contamination and effects on wildlife, including adaptive responses. However, metals have been studied much less, although previous sediment sample analyses from Pescara Creek on the Elizabeth River contained metal concentrations above NOAA thresholds. Pescara Creek provides a unique site to enable understanding of higher levels of metal

contamination as well as established moderate organic contamination. Atlantic Killifish inhabiting metal contaminated sites in the Elizabeth River provide a natural experimental model to compare tissue specific variation in metal accumulation, relative to environmental metal levels.

**Objective:** Our objective was to analyze metal concentrations in water, sediment, and Atlantic Killifish tissue samples from Pescara Creek compared to the King's Creek reference site.

**Methods:** Samples were collected at both sites in October 2021. For each site, 10 male and 10 female fish were collected. Nine sediment samples were collected as well as one filtered and one unfiltered water surface water sample for each site. Fish were dissected to collect brain, liver, kidney, gills, heart, and muscle tissue. Samples were freeze-dried and processed using acid digestion, and then analyzed using ICP-MS to obtain concentration values for lead, cadmium, arsenic, selenium, mercury, manganese, vanadium, zinc, and copper.

**Results:** Based on data collected to date, accumulation differs by tissue. In addition, there were differences between the two sites for some metals; notably, lead levels were 2-5 times higher in Pescara Creek tissues, notably gills. In water sample analysis there were elevated concentrations of copper, zinc, manganese, and lead compared to the reference site. Some of these metals may likely be bound to dissolved particles in the water.

**Conclusions:** Based on preliminary results, it appears that Pescara Creek killifish accumulate higher levels of potentially harmful metals than reference site fish. This can potentially be attributed to both sediment and aqueous concentration of the metals. Future work includes testing how this chronic exposure impacts their response to other stressors.

**Relevance**: Metal exposure despite extensive study is still prevalent in many ecosystems and understanding how they affect local populations of both humans and wildlife is important.

## 2.10 Pelagic and Benthic Harmful Algal Blooms (HABs): The Detection, Fate, Effects, Monitoring, and Management of Blooms

### 2.10.T-01 Strategies for Preventing, Managing, and Responding to Harmful Cyanobacterial Blooms.

***Ben Holcomb**, Utah Division of Water Quality*

Cyanobacterial blooms are a common occurrence across North America and elsewhere. In some cases, they may produce potent toxins harmful to people and animals. Harmful cyanobacterial blooms (HCBs) can negatively impact drinking water systems, recreation, aquatic habitat, irrigation, and property values. During bloom events immediate response and communication are very important. Common questions arise such as: how do I collect a sample; which lab analyses should be employed; how do I interpret the results and communicate to stakeholders; can these blooms be treated or prevented? The answers to these questions are not readily available in one place.

The Interstate Technology and Regulatory Council's (ITRC) HCB team (comprised of federal, state, academic, local, and private experts across the US) has developed guidance documents, interactive tools, and videos for state regulators and others responding to both planktonic and benthic HCBs into a readily available web portal. The information is intended to help you select monitoring, communication, in-lake management, and nutrient reduction approaches that are suitable for use in your water body and community. The portal provides introductory information such as cyanobacteria basics, toxin analyses, identification by way of macroscopic and microscopic imagery, and videos. No management strategy will be effective in every water body or watershed. Local characteristics, environmental triggers, and human activities must all be considered to use HCB prevention and management strategies successfully. Monitoring is key to identifying what supports cyanobacterial growth and evaluating whether your strategies have been successful. Consistent, informative, effective communication helps protect your community and builds supports for implementation of your resource plan. Because bloom response requires rapid action, time-critical communication information was categorized for ease of use. The web portal provides in-depth case studies, citations, and resource links to provide the information, resources, and interactive tools to help you build a water resource or watershed management plan that is appropriate for your local needs.

**2.10.T-02 Planktonic Growth Potential of Overwintering Cyanobacteria in Sediment from Freshwater Reservoirs Impacted by Harmful Algal Blooms**

***Alyssa Calomeni***[1]*, Andrew D McQueen*[1]*, Ciera Kinley-Baird*[2] *and Gerard Clyde, Jr.*[3]*, (1) U.S. Army Engineer Research and Development Center, (2) Aquatic Control, (3) U.S. Army Corps of Engineers*

Freshwater cyanobacteria causing harmful algal blooms (HABs) can overwinter in sediments as dormant cells (akinetes or vegetative colonies) and contribute to the formation of planktonic blooms. Targeting overwintering cells in sediments of HAB-impacted freshwater reservoirs may provide a viable approach to mitigate the timing, intensity and severity of seasonal blooms. However, this is a novel strategy and there are limited data and resources to inform preventative management. Therefore, the overall objective of this study was to identify and illustrate relevant data needs to support identification of sites that contain overwintering cells with the potential to form planktonic HABs. To achieve this, sediment samples were collected, and overwintering cells were enumerated from three HAB-impacted freshwater reservoirs in the central US as pertinent examples. To inform overwintering cell viability and growth potential, laboratory incubation studies were developed based on peer-reviewed literature of environmental conditions (e.g. temperature, light, nutrients) suitable for overwintering cyanobacteria growth. Overwintering cells were present in sediments at all three of the freshwater reservoirs sampled, with 85% of sites (n=13) containing akinetes or overwintering colonies in sediments and 54% of sites (n=13) with a growth potential producing problematic cell densities (>100,000 cells/mL) in the planktonic phase. These data indicate that a monitoring approach should consider multiple lines of evidence: 1) presence and density of overwintering cyanobacteria, 2) planktonic growth potential as informed by laboratory incubation studies, and 3) environmental conditions at the sediment water interface (e.g. light intensity and attenuation, temperature, nutrient conditions, and mixing potential) to predict growth risk and prioritize locations for preventative management.

**2.10.T-03 Spatiotemporal Occurrence and Water Quality Hazards of Common Cyanobacterial Toxins in Warm-Monomictic Reservoirs Located Across a Pronounced Annual Rainfall Gradient**

***Kevin Stroski***[1]*, Daniel L. Roelke*[2]*, Crista M. Kieley*[2]*, Royoung Park*[2]*, Kathryn L. Campbell*[2]*, Hagen Klobusnik*[2]*, Jordan R. Walker*[2]*, Sierra E. Cagle*[2]*, Jessica M. Labonte*[2] *and Bryan W. Brooks*[1]*, (1) Baylor University, (2) Texas A&M University*

Occurrence of harmful algal blooms (HABs) have been increasing in magnitude and frequency due to anthropogenic factors such as eutrophication and climate change. These blooms can present important risks to public health and environment due to toxins produced when HAB events occur. Here, we examined occurrence profiles of common cyanotoxins, including five common microcystins (MCs), cylindrospermopsin, anatoxin-a, saxitoxin and nodularin in twenty reservoirs spatially distributed across a pronounced annual rainfall gradient in southcentral USA. In contrast to routine surface water quality monitoring approaches, these twenty systems were sampled in both spring and summer, at different depths and at mid-lake and near dam lacustrine locations. We then employed probabilistic environmental hazard analyses to examine whether cyanotoxins exceedances occurred and if these exceedances varied spatiotemporally. Highest concentrations were observed during spring in two reservoirs receiving the lowest annual rainfall. Microcystin-LR was the most common congener detected, but microcystin-YR was detected at the highest concentrations with many observations above the California human recreation guideline value (800 ng/L). In most instances, surface and bottom water samples showed similar concentrations at matching sites, indicating that these toxins may be present throughout the water column rather than only at the surface, where phytoplankton concentrations are higher and therefore where routine monitoring occurs. In summary, 11% of locations sampled in the spring exceeded total microcystin levels of 800 ng/L, while 4% of mid-lake sites, 3% of surface and bottom locations, 2% of near dam sites, and none of summer samples were found higher than this regulatory value. Our observations have implications for routine water quality monitoring practices, which traditionally focus on collection of surface samples (e.g., 0.3 m) during summer near the impoundment of these reservoirs and indicate spatiotemporal surveillance efforts are necessary to characterize toxin exposures and risks when HAB events occur.

**2.10.T-04 Harmful Cyanobacterial Aerosolization Dynamics in the Airshed of a Eutrophic Estuary**
***Haley Plaas***[1]*, Karsten Baumann*[1]*, Ryan Paerl*[2]*, Kimberly Popendorf*[3]*, Colleen Karl*[4]*, Nathan S. Hall*[1]*, Malcolm Barnard*[1]*, Naomi Chang*[1]*, Hwa Huang*[2]*, Nathaniel P. Curtis*[2]*, Olivia L. Mathieson*[2]*, Joel Sanchez*[2]*, Daniela Maizel*[3]*, Amy N. Bartenfelder*[1]*, Jeremy Braddy*[1]*, Karen Rossignol*[1]*, Randy Sloup*[1] *and Hans Paerl*[1]*, (1) University of North Carolina at Chapel Hill, (2) North Carolina State University, (3) University of Miami, (4) Chowan Edenton Environmental Group*
In addition to obvious negative effects on water quality, recent work suggests that harmful cyanobacterial blooms (CHABs) impact air quality via emissions of cyanobacterial cells, cyanotoxins, and other emerging biogenic compounds. Several toxigenic cyanobacterial genera and cyanotoxins have been detected in aerosol, but the associated environmental controls and human exposure risks remain largely unknown. Accordingly, this study aimed to 1) investigate the occurrence of microcystin (MC) and cyanobacterial communities in particulate matter <2.5 µm in diameter ($PM_{2.5}$), 2) elucidate environmental conditions promoting their aerosolization, and 3) evaluate other apparent associations between CHABs and regional air quality in the airshed of the Chowan River, a eutrophic estuarine system in eastern North Carolina impacted by recurring CHABs. In summer 2020, during peak CHAB season, continuous $PM_{2.5}$ samples and interval water samples were collected for targeted analyses of cyanobacterial community composition and MC concentrations. Supporting air and water quality measurements were made in parallel to contextualize findings and permit statistical analyses of environmental factors driving changes in CHAB-derived aerosol. Several aquatic CHAB genera which were genetically traced to water samples were identified in $PM_{2.5}$, including *Anabaena, Aphanizomenon, Dolichospermum, Microcystis, Pseudanabaena,* and *Tolypothrix.* Cyanobacterial enrichment in $PM_{2.5}$ was indistinctive at the subspecies level, but bloom state was an important factor in the enrichment of all CHAB cells in $PM_{2.5}$. MC production was low throughout the study, but a mixed assemblage CHAB dominated by *Dolichospermum* occurred from late-June to mid-July. In association with the CHAB, the median $PM_{2.5}$ mass concentrations increased to 10.5 µg $m^{-3}$ (IQR = 4.55), significantly above the non-bloom background of 5.81 µg $m^{-3}$ (IQR = 5.48) (W = 1465, $p < 0.001$). Results underscore the need to better investigate the role that freshwater harmful algal blooms, specifically HABs, play in regional air quality and subsequent respiratory health risk.

**2.10.T-05 Occurrence of Harmful Algal Blooms (HABs) in Urban Environments**
***Heath Mash****, Laura Webb, Marcie Tidd, Jingrang Lu, Morgan McNeely, Regina Klepikow, Maddie Castro, Bill Schroeder and Ryan Monahan, U.S. Environmental Protection Agency*
Algal blooms are a natural component of aquatic ecosystems. However, these are often composed of microorganisms known as cyanobacteria, which may produce toxins that can pose a significant threat to human, animal and ecological health through the contamination of waterways used for recreational purposes as well as drinking water supplies. Many studies have focused on water bodies that have had significant agricultural impact from nutrient runoff that tend to be located in rural locations outside major urban centers. In contrast, water bodies located in urban environments tend to be less impacted by agricultural practices; instead, water they are impacted by more industrial/residential activities or sewage runoff. These waters include smaller drainage basins and may either have shorter residence times due to their smaller size relative to larger rural water bodies or are stagnant.

This project investigates lakes located in three major urban centers in the United States: Cincinnati OH, Kansas City MO/KS and Denver CO. This study aims to determine if there are water quality parameters that are unique to these lakes, and to investigate the correlation between urban watershed characteristics and cyanobacterial blooms. Water samples were taken over the course of one recreational season along with the deployment of time-weighted passive samplers. The study involved the identification of cyanotoxins and cyanobacteria, measurement of urban contaminants such as metals, pesticides, semi-volatile organics and nutrients, and physical parameters.

**2.10.T-06 Investigating Effects of Developmental Domoic Acid Exposure on Neuroimmune Cells in Larval Zebrafish (Danio rerio)**

***Alia S Hidayat***[1,2] *and Neel Aluru*[1,3]*, (1) Woods Hole Oceanographic Institution, (2) MIT-WHOI Joint Program, (3) Woods Hole Center for Oceans and Human Health*

Domoic acid (DA) is a neurotoxin produced by the diatom *Pseudo-nitzschia* that can accumulate in shellfish and cause amnesic shellfish poisoning (ASP). Clinical signs of DA toxicosis such as disorientation, seizures, and uncoordinated movement have been reported in several wildlife species and in humans. Though studies in animal models suggest that early developmental stages are particularly susceptible, the cellular mechanisms of developmental DA toxicity are not well understood. We hypothesize that, as the resident immune cells of the central nervous system (CNS) and primary responders to CNS injury, microglia are activated by DA exposure and that this could have long-term effects on adult health and behavior. We utilized the transgenic zebrafish line Tg(*mpeg1*:mCherry), which labels microglia, to visualize and quantify changes in cell abundance and morphology in response to exposure. Developing zebrafish were exposed to various concentrations of DA (0, 0.06, 0.1, 0.3ng) via hindbrain ventricle injection at two developmental stages (2 and 4 days post fertilization) to identify windows of susceptibility. Significant differences in sensitivity were observed between the two developmental time points. Exposure to 0.3ng DA at 4dpf caused activated microglial morphology, alongside cardiac and yolk sac edema, brain necrosis, lack of swim bladder, and spinal curvature, suggesting 0.3ng is acutely toxic to developing embryos. In contrast, 0.1ng DA exposure to 2dpf (but not 4dpf) embryos did not cause overt toxicity, but induced an activated microglial morphology at 4 hours post exposure (hpe) and an increase in microglial abundance at 24hpe. Furthermore, assessment of cell death with acridine orange suggests that this activation occurs independent of acute neurotoxicity. These results demonstrate that developmental DA exposure causes microglial activation, which could have long-term health effects given the critical role of microglia in CNS development. We are currently testing the hypothesis that developmental exposure may have persistent impacts on microglial abundance and increase blood brain barrier permeability. Additionally, we are currently raising these fish to adulthood to investigate the potential long-term behavioral impacts of developmental DA exposure. This work is supported by an NSF Graduate Research Fellowship (Award #112374) and the Woods Hole Center for Oceans and Human Health (NIH P01ES028938 Diversity Supplement and NSF OCE 1840381).

**2.10.T-07 Comparative Understanding of the Developmental Neurotoxic Effects of Chiral Cyanotoxin Anatoxin-a in Two Common Fish Models**

***Lea Marie Lovin***[1]*, Laura M. Langan*[1]*, Sujin Kim*[1]*, Raegyn B. Taylor*[2]*, Kendall Rose Scarlett*[1]*, Kevin Chambliss*[1]*, Saurabh Chatterjee*[3]*, Thad Scott*[1] *and Bryan W. Brooks*[1]*, (1) Baylor University, (2) U.S. Department of Agriculture, (3) University of South Carolina*

Harmful algal bloom toxins present risks to public health and the environment. The cyanotoxin anatoxin-a (antx-a) is a globally found, potent neurotoxin. To understand this compound in context of previous literature we conducted a meta-analysis in which we compared environmental occurrence data to international guideline values to create probabilistic hazard assessments and collated toxicity data which we found to be was lacking, especially in terms of enantiomer specific and sublethal responses. To help address these gaps, we studied larval fathead minnow and zebrafish behavioral and molecular responses (gene expression, transcriptomics, and proteomics) following exposure to either (±) or (+) antx-a at environmentally relevant, analytically verified concentrations based on the meta-analysis (10-1500 µg/L) and a higher concentration of 3000 µg/L to study mechanistic change. We found duration and distance of fathead minnows swimming at the highest speed was significantly lowered by (±) antx-a in all but the lowest treatment level, while the opposite occurred with zebrafish, which showed increased distance, duration, and changes in movement at the highest speed, though not significant. We examined gene expression focusing on genes related to neurotoxicity, oxidative stress, DNA damage, and hepatotoxicity. While there was little change in zebrafish, fathead minnows showed expression changes related to neurotoxicity and oxidative stress. This suggests fathead minnows may be more sensitive to this toxin based on these endpoints. In studying purified (+) antx-a, the only enantiomer naturally produced, we observed high mortality in fathead minnows >500 µg/L and largely refractory, lowered movement in zebrafish for most endpoints. Which corroborates our sensitivity findings with (±) antx-a and highlights the increased toxicity of the purified enantiomer. We are currently running gene expression analysis on these samples with an expanded profile based on tandem proteomics experiments. We plan to create full data independent proteomics profiles and transcriptomic profiles using 3rd gen nanopore sequencing. Understanding of cyanotoxin effects in different species under environmentally relevant conditions is necessary to support robust assessments and management of algal blooms and to anticipate related health concerns.

**2.10.T-08 Assessing the Effects of Lyngbya wollei Toxins on the Adult Fathead Minnow (Pimephales promelas)**

***Margaret A Carson***, *Tryston Metz, John Ferry and Geoffrey I. Scott, University of South Carolina*

Harmful algal blooms (HABs) are naturally occurring, excessive growths of algae that have the potential to produce toxins harmful to water and air quality, aquatic species, and humans. Key forcing factors for the development of HABs include climate change, nutrient enrichment, urbanization, and other anthropogenic activities. Many HAB species are invasive and/or opportunistic and take advantage of altered habitat conditions and the HAB growth in Lake Wateree, South Carolina is no exception. Researchers have observed extensive blooms of the benthic cyanobacterial algal mat, *Lyngbya wollei* (*Microseira wollei*) in Lake Wateree, South Carolina since 2012. This study aimed to address the potential hazards posed by these HAB toxins to aquatic species. Acute 96-hour toxicity tests were conducted on adult Fathead Minnows (*Pimephales promelas*) using nominal concentrations of freeze-dried, live, and air-dried algae. Algae samples were collected from one site on Lake Wateree across 3 months and transferred back to the lab. Levels of *Lyngbya wollei* toxins were quantified, using high resolution mass spectrometry. A total of 3 replicates/treatment/site were used and the mortality over time for the test was the endpoint of each bioassay. Laboratory toxicity tests indicated that the live algae was not toxic, but both the air-dried algae and freeze-dried algae were acutely toxic. The mortality results varied based off concentration and month of algae collection. Median lethal concentration (LC50) for each of the acute toxicity tests were determined and will be discussed. Results of field toxicity tests will be discussed. Results show that acute exposure to Lyngbya wollei has varying levels of effect depending on the physical state of the algae (live, freeze-dried, or air-dried). This suggests that there are varying amounts of toxin dissolved into water, depending on if the cell is fully lysed or not. This information can be used to enhance HAB management strategies in freshwater bodies that are impacted by Lyngbya wollei. Additionally, freshwater managers can utilize toxicity data to determine whether areas are safe to use for contact recreation or drinking water according to water quality standards. Finally, this information can be used to help lake residents and users who are concerned about the presence of HAB species and the potential impacts for human exposure to the toxin when using the lake. Education materials are being developed about Lyngbya wollei from this study

**2.10.P Pelagic and Benthic Harmful Algal Blooms (HABs): The Detection, Fate, Effects, Monitoring, and Management of Blooms**

**2.10.P-We017 Development of a Microcystis aeruginosa culture method to produce sufficient amounts of microcystin to conduct multispecies acute and chronic toxicity tests.**

***James M. Lazorchak***[1]*, Susanna DeCelles*[2]*, Alex Kascak*[2]*, William Thoeny*[2]*, Nicholas Dugan*[1]*, Toby Sanan*[1]*, Ian Struewing*[1]*, Joel Allen*[1] *and Jingrang Lu*[1]*, (1) U.S. Environmental Protection Agency, (2) Pegasus co US EPA, Cincinnati*

There is a lack of information to estimate safe exposure levels to toxins produced by cyanobacteria for freshwater aquatic life. The uncertainty in concentrations and purity of standards for cyanotoxins, and potentially their cost, challenge their use for conducting acute and chronic toxicity tests. To address this, different approaches to culture cyanobacteria for toxicity assessments were compared. Microcystin-producing *Microcystis aeruginosa* with confirmed *mcy* genes by qPCR and RT-qPCR was used in these cultures. Initial tests were conducted using lysates obtained by removing cells from culture media by centrifugation and then lysing the cells by freeze thawing them three times. Early culture efforts resulted in a range of total microcystin concentrations (37-73 µg/L) that varied in cell density (3.73-4.42 x$10^6$ cells/mL) and similar age (≈150 days). Another method using a different vessel format (8 flasks of different ages combined into 21 L), similar age of culture (≈150 days), and a cell density of 1.75 x$10^6$ cells/mL, resulted in a microcystin concentration of 885 µg/L. This culture vessel format was repeated and resulted in a cell density of 1.38 x$10^6$ cells/mL and a lysate concentration of 426 µg/L. Due to the variability in toxin yields, an approach was taken to grow *M. aeruginosa* cultures up to a stationary phase in 250-mL and 1-L flasks, then test for toxin concentrations using ELISA. However, cell extracts were too low to be suitable for adequate aquatic toxicity testing, with microcystin concentrations of 25 µg/L. Finally, an aerated culture under nutrient replete conditions with lower temperature and illumination in two carboys containing 14 L in each, a 28-day cell density of 9.5 x$10^6$ cells/mL, resulted in a lysate microcystin concentration of 517 µg/L. These culture conditions were repeated, and the lysate microcystin concentrations were tested. Acute toxicity tests conducted with *Ceriodaphnia dubia*, *Neocloeon triangulifer*, *Hyalella azteca,* and larval *Pimephales promelas* using microcystin lysate did not cause significant mortality at concentrations as high as 74 µg/L. For chronic tests, IC25 for total microcystins for *C. dubia*, *P. promelas*, *H. azteca*, *N. triangulifer* were 8.9, 74.0, 408.9, and 10.1 µg/L, respectively. Results of continued testing of the new culture methods and acute and chronic toxicity tests will be presented.

**2.10.P-We018 Immuno-Affinity Magnetic Beads For Cyanotoxin Extraction And Clean Of Biological Samples**

***Leah Ruth**, Rachael Lathers, Graham Yearwood and Fernando Rubio, R&D, Eurofins Abraxis, Inc.*

Cyanotoxins are produced by cyanobacteria which include neurotoxins such as anatoxin-a (ATX-a) and heptotoxins such as microcystins (MCT). Cyanobacteria are found in lakes, rivers, and oceans around the world. Under eutrophic conditions, they reproduce exponentially to form blooms. In some cases, these blooms produce cyanotoxins in concentrations that pose a significant threat to human and aquatic life through contamination of recreational, drinking and irrigation water supplies, as well as accumulation in food sources such as fish and shellfish tissue.

Given its prevalence and toxicity, the US EPA has published health advisory levels for the hepatotoxin microcystins in both drinking water and recreational water. Many US states have also adopted guidance for microcystins as well as other cyanotoxins including the potent neurotoxin, anatoxin-a. The present study seeks to evaluate a simple method for sample clean-up that can be used on biological samples prior to cyanotoxin analysis to help assess the risk of cyanotoxin exposure.

Immunoaffinity magnetic beads (IMB) are synthesized by conjugating monoclonal antibodies (mAbs) with magnetic beads, separation is achieved by the use of a strong magnet. The interactions between antigen and antibody are highly specific, so the IMB clean-up method possesses high specificity and selectivity. In addition, the large specific surface area and the dispersion properties of IMB greatly shorten equilibrium time and increase interactions between the sorbent and target, which results in a higher extraction capacity and detection sensitivity. Depending on the elution volume used, a sample concentration of 5-10X can be achieved.

In the present study, a pre-treatment and concentration method was developed for highly efficient and selective isolation and concentration of microcystins and anatoxin-a on the basis of anti-MCT and anti-ATX-a IMB. The factors affecting the incubation with the IMBs and elution of MCT and ATX-a from the IMBs were meticulously tuned for efficient and selective isolation and concentration of the toxins from urine and serum samples for downstream analysis such as by ELISA or LC/MS.

The overall procedure takes approximately forty-five minutes (multiple samples can be extracted in parallel), demonstrating the simple character of the proposed clean-up/concentration procedure. Under the optimal conditions, the developed method has good linearity, LODs, LOQs, accuracy, precision, and selectivity.

### 2.10.P-We019 Benthic Cyanobacterial Blooms in Stormwater Ponds

***Avery Tatters***[1]*, Courtney Kapczynski*[2]*, Raphael Kudela*[3]*, Stuart Oehrle*[4]*, Wendy Stangman*[2]*, Jessica Aukamp*[1] *and Yongshan Wan*[1]*, (1) U.S. Environmental Protection Agency, (2) University of North Carolina, Wilmington, (3) University of California, Santa Cruz, (4) NKU*

Extensive cyanobacteria-dominated mats were observed during late summer of 2021 within a stormwater retention pond network in northwest Florida. Common in residential communities across the region, such ponds may be routinely visited by humans, domestic and wild animals, and often contain a variety of aquatic life. Accordingly, we investigated the temporal heterogeneity of potentially toxic cyanobacteria, associated toxins, and 'under the radar' bioactive peptides throughout the fall at several sites within the network. Multiple toxin classes were detected in whole water and SPATT samples using targeted analysis including levels that far exceeded national recreational guidelines. Non-targeted analysis also revealed a collection of potentially unknown cyanobacteria-associated compounds that, along with known toxins, oscillated with periods of heavy rainfall and temperature variation. Our results suggest that the benthic proliferations and associated toxins are correlated with an intended function of the stormwater treatment network, nutrient removal. Furthermore, these retention ponds may serve as a consistent source for cyanobacteria biomass accumulation that is transported downstream in the form of cells for colonization and cyanotoxins to estuarine waters.

### 2.10.P-We020 Toxic Benthic Cyanobacteria as an Emerging Ecological and Public Health Threat in Virginia

***Samantha Mohney*** *and Scott Glaberman, George Mason University*

Toxic benthic cyanobacterial harmful algal blooms (cHABs) are a growing human and environmental health concern due to their negative impacts on water quality, drinking water contamination, and public health. A recent trend has emerged showing a rise in the number of reported benthic cHAB events occurring in the eastern seaboard of the U.S. We investigated the appearance and proliferation of the toxic benthic cyanobacterium, *Microseira* (formerly *Lyngbya*) *wollei*, within the Potomac River and its tributaries, and examined its seasonality, magnitude, and growth dynamics. We found a significant upward trend for biomass and growth through the summer months, and well into October. Our current work focuses on understanding the relationship between spatiotemporal dynamics of *M. wollei* and toxin production in relation to changing environmental conditions in the Potomac River. To accomplish this, we are using genetics to compare benthic cHABs in the Potomac River to other areas of global occurrence. We are also examining toxin production in these cHABs in terms of both quantity and toxin type. The overall goal is to understand the drivers of cHAB occurrence and toxin production as a function of climate change and water quality. Findings will help guide cHAB monitoring and management efforts.

### 2.10.P-We021 Ecological Toxicity of Cyanotoxins: Development of an Evidence Map using ECOTOXicology Knowledgebase Systematic Protocols

***Jennifer Olker***[1]*, Aabir Banerji*[1]*, Kasey Benesh*[1]*, Brian Kinziger*[2]*, Traci Scott*[2]*, Travis Karschnik*[2]*, Timothy Feist*[2]*, Anne Pilli*[2] *and Dale J. Hoff*[1]*, (1) U.S. Environmental Protection Agency, (2) General Dynamics Information Technology*

Cyanotoxins associated with Harmful Algal Blooms (HABs) can be deadly to humans, pets, and livestock. Impacts of cyanotoxins on other organisms (e.g., microbes, plants, and wildlife) are not well characterized. Our goal was to comprehensively and systematically assemble available evidence for ecological effects of

cyanotoxins using the well-established curation protocols of the ECOTOXicology Knowledgebase (ECOTOX, www.epa.gov/ecotox) and report the extent, distribution, and types of evidence. The initial literature search encompassed cyanotoxins produced by the most common HAB-forming cyanobacteria in the U.S.: microcystin-LR, microcystin-RR, anatoxin-a, cylindrospermopsin, saxitoxin, and lyngbyatoxin-a. This list was expanded to include several cyanotoxins known to be commonly detected or associated with coastal or marine systems and then further expanded to include over 100 cyanotoxins documented in publications and government reports. The 7,500 references retrieved were screened at the title and abstract level. Full-text review of the 1,100 that met inclusion criteria for ECOTOX (e.g., verifiable species and CASRN, control reported) yielded ~600 references with relevant ecological toxicity data. Data extraction is underway to capture all pertinent study details (species, chemicals, test methods, and toxicity results) and incorporate them into the ECOTOX public website. To date, toxicity results have been extracted for 28 cyanotoxins, with >60% of the results pertaining to microcystins. Studies include testing for effects in aquatic and terrestrial vertebrates, invertebrates, and plants, with almost 50% of the reported toxicity results in fish and notable under-representation for several species groups such as amphibians and birds. 58% of references (but only 20% of records) measured traditional growth/reproduction/mortality toxicity endpoints, with 70% of references (60% of records) also including measurements for biochemical or genetic effects. These results will be presented in an evidence map for ecological toxicity of cyanotoxins with the extent of empirical data for aquatic and terrestrial organisms by species, types of effects, and cyanotoxin class. *This abstract does not necessarily reflect US EPA policy.*

**2.10.P-We022 Characterizing Benthic Cyanobacteria and Their Toxicity in the Finger Lakes of New York State**

***Abby Webster**[1], Lisa B. Cleckner[2], Gregory L. Boyer[1] and N. Roxanna Razavi[1], (1) SUNY College of Environmental Science and Forestry, (2) Hobart and William Smith Colleges*

The United States are behind the rest of the world in studying benthic algae, particularly in lake littoral zones which are known for their high rates of primary production. As planktonic harmful algal blooms (HABs) continue to receive most of the attention, benthic algae proliferations remain far understudied and are an increasing threat to water quality. The Finger Lakes of New York State are no exception to this and are an ideal location to study benthic blooms as the lakes range in size and trophic status, and all eleven Finger Lakes have experienced planktonic cyanobacterial HABs in recent years. We present two major questions: 1) What makes up the cyanobacterial community of nearshore benthic algae in the Finger Lakes, and 2) Are they toxic? These questions have gone unanswered due to the lack of standardized sampling and monitoring methods for benthic algae in lakes. We developed a standardized method to answer these questions in three Finger Lakes of meso-oligotrophic, mesotrophic, and eutrophic status. Periphyton samples will be collected from each lake throughout the growing season, identified to the highest taxonomic resolution possible through microscopy, subsequently sent for metagenomics, and tested for cyanotoxins using LC-MS/MS. Method testing in 2021 using artificial substrates and an *in situ* probe indicated that attached cyanobacteria increase in relative proportion to diatoms (dominant) and green algae (least abundant) as the summer progresses but are present at all sites during the entire sampling season. Differences exist among these lakes in the planktonic cyanobacteria communities and their toxicities, and we expect this to be true in benthic algae as well. These data will better our understanding of benthic algae proliferations in temperate lakes and to what extent they may be harmful.

**2.10.V Pelagic and Benthic Harmful Algal Blooms (HABs): The Detection, Fate, Effects, Monitoring, and Management of Blooms**

**2.10.V-01 Biodegradation of Microcystins Using mlr Gene Cluster: A Review on Recent Advances Based on Bibliometric and Content Analysis (1994 – May 2022)**

***Qinyi Wang**[1], Youran Zhao[1] and Qin Ding[2], (1) Nanjing Foreign Language School, China, (2) Southeast University, China*

Microcystins, a group of cyclic-heptapeptide hepatoxins, are among the most potent varieties of cyanotoxins.

Their presence in aquatic systems has become a major threat to environmental and ecological stability, as well as to public health. Since the first investigation of microcystinase and the corresponding *mlr* gene cluster in 1996, the genetic approach to microcystins degradation has become a prevalent focus. This study retrieved and evaluated 91 articles from Scopus (1994 – May.2022) on the decomposition of microcystins specifically with *mlr* genes, through bibliometrics and content analysis to illustrate leading contributors and up-to-date research hotspots of this subtopic with urgent demand. Furthermore, notable outcomes and a future outlook was summarized as reference for researchers. Results indicated that utilizations of *mlr* genes and host bacteria are novel advances, as the average year of appearance of key words '*mlr* gene' 'genetics' 'bacterial protein' were approximately 2017. China was the most productive with 43 publications, followed by the United States, Japan and Australia with 11 documents each. Journals *Chemosphere* and *Toxins* were key publishers with 9 articles each. Three topics: separation from natural strains, determination of degradation pathway and proposal of implementations, were the current keynotes of *mlr* genes research. Heterologous expression has clarified respective metabolic roles of MlrABCD. Recently, there has been increasing interest in linearized-microcystins: the use of MlrA, the most effective enzyme for microcystins detoxification, is commonly followed by physical or chemical methods to treat the less toxic by-product. Such interdisciplinary approaches, as well as the optimization of *mlr* expression in recombinant bacteria for bioaugmentation, have been found promising and should be given more recognition in future studies. This work outlines the current research status of using *mlr* genes for biodegradation, provides insight on potential improvement opportunities, and suggests a future direction towards *mlrA*-based composite bioremediation.

**2.10.V-02 California's Harmful Algal Bloom-Related Illness Workgroup Identifies Atypical Routes of Potential Human Exposure**

***Beckye Stanton***[1]*, Shannon Murphy*[1]*, Susan Paulukonis*[2]*, Isadora Nogueira*[2]*, Jeff Fowles*[3]*, Carly Nilson*[4]*, Marisa Van Dyke*[4] *and Jenna Rinde*[5]*, (1) California Office of Environmental Health Hazard Assessment, (2) Public Health Institute, Tracking California, (3) California Department of Public Health, (4) California State Water Resources Control Board, (5) California Department of Fish and Wildlife*

The California Interagency Harmful Algal Bloom (HAB)-related Illness Workgroup investigates and tracks potential HAB-related illnesses in humans and animals throughout California. All potential illness reports submitted to the workgroup undergo an evaluation of the available environmental and health-related information. As part of these efforts, the workgroup investigated potential freshwater and marine HAB-related illness reports in humans exposed through several pathways not typically considered. We received several reports of individuals who consumed water from natural freshwater lakes and streams with cyanobacterial HABs after using portable water treatment/filtration devices. The workgroup also investigated multiple illness reports following dermal exposure to marine waters during dinoflagellate blooms. Future research needs identified include portable drinking water device effectiveness with cyanobacteria and cyanotoxins in raw water sources and the mechanisms of dermal allergic or irritant responses following water contact during marine HABs. In addition, further consideration of these atypical pathways of potential exposure in illness investigations, HAB monitoring, and public health messaging could ultimately improve public health interventions and guidance.

**2.11 Quantifying the Fate and Effects of Metals: Balancing Complexity with Practicality**

**2.11.T-01 Seasonality of insect-mediated Hg flux out of human-made ponds**

***Madeline Pratt Hannappel***[1]*, Matthew M. Chumchal*[2]*, Ray Drenner*[2]*, Lexi Freeman*[2] *and Jim Kennedy*[1]*, (1) University of North Texas, (2) Texas Christian University*

Methylmercury (MeHg) produced in aquatic systems can be transported to terrestrial predators by emergent aquatic insects (referred to as insect-mediated Hg flux). Human-made ponds can be important sources of insect-mediated Hg flux to terrestrial food webs, but few studies have assessed the Hg flux out of human-made ponds. In this study, we assessed insect-mediated Hg flux from 6 human-made ponds at the LBJ National Grasslands in

North Texas. Emergent aquatic insects were collected from ponds using floating emergence traps deployed for 4- to 9-day intervals from March to July of 2018 (total 61 days sampled). The 6 ponds sampled included one temporary pond that dried during the sampling period, one permanent pond with fish, and 4 semipermanent fishless ponds. We calculated Hg flux for each insect taxa as emergent insect biomass x total Hg concentration (THg) per $m^2$ per day. Insect-mediated Hg flux out of ponds ranged from 0.5-5.6 ng/$m^2$/day (mean 3.4). The emergent insect taxa contributing to Hg flux varied among ponds. Because odonates (dragonflies and damselflies) had both a higher biomass and higher THg concentrations compared to other emerging taxa, they represented the largest proportion of Hg flux out of ponds and strongly influenced seasonal Hg flux more than other taxa. However, non-odonates made up the vast majority of individual insects emerging from all ponds (N/m2/day; mean 98%). In ponds without fish, chironomids (Chironomidae: Tanypodinae, Orthocladinae, and Chironominae) made up the highest proportion of emergence (mean 81%) but only made up 32% of the Hg Flux. We detected a significant effect of date on Hg flux from 5 of the 8 insect taxa and aggregate Hg flux, with aggregate Hg flux peaking between May and June and individual insect taxa peaking at different times during the season. These results suggest there is a seasonal pulse of Hg flux out of human-made ponds peaking mid-summer and that Hg flux differs among emergent insect taxa. There likely is an effect of pond type on the taxa and number of insects emerging and, subsequently, the aggregate Hg flux out of ponds.

**2.11.T-02 Plastic's Role in Mercury Transport Throughout the Matagorda Bay System**
*Jessica Myers[1], Jacob Oster[1], Oluniyi O. Fadare[1], Stephanie Lewis[1], Jessica Dutton[2] and* ***Jeremy L. Conkle[1]****, (1) Texas A&M University, Corpus Christi, (2) Texas State University*
Plastic debris is ubiquitous in Texas' coastal bays, where there are heavy industrial footprints. The Matagorda Bay system is a worst-case scenario where there are industrial plastic discharges, post-consumer plastics, and a mercury Superfund site. This study aimed to assess the role of plastic debris in mercury transport throughout the Matagorda Bay system. We will address our aim by quantifying mercury concentrations on plastic debris, both pre-production materials and post-consumer litter from the shorelines of Matagorda Bay. Additionally, the stomachs of southern flounder (*Paralichthys lethostigma*), red drum (*Sciaenop ocellatus*), and speckled trout (*Cynoscion nebulosus*) will be examined for microplastic presence while their fillets are analyzed for total mercury. Plastic material types will be verified using Fourier-Transform Infrared Spectroscopy, and total mercury concentration will be quantified using a Direct Mercury Analyzer. The results will examine mercury concentrations relative to plastics properties and distance from the Superfund site and mercury present in the fillet of the three fish species studied. This work will improve our understanding of plastic and mercury interactions to better manage this ecosystem's health while also educating stakeholders in the region about this pollution.

**2.11.T-03 The Coupled Trophic Transfer of Selenium and Mercury in a Large River**
***Raul Flamenco[1]****, Travis S. Schmidt[2], James Roberts[2], Craig Stricker[2], Collin Eagles-Smith[2] and Jessica Brandt[1], (1) University of Connecticut, (2) U.S. Geological Survey*
Mercury (Hg) is a naturally occurring contaminant that bioaccumulates and biomagnifies in aquatic food webs. Potential consequences of excess Hg exposure include neurotoxicity, teratogenesis, and mortality, especially in high trophic level consumers. Some studies suggest that selenium (Se) can mediate Hg methylation, bioaccumulation, and toxicity, yet uncertainties remain in the consistency of this phenomenon across taxa and ecosystems. One emerging hypothesis from this literature is that the availability of Se may interfere with Hg trophic transfer by reducing Hg bioavailability or enhancing Hg detoxification and elimination. To address the related question of whether Se limits Hg trophic transfer from prey to predator, we draw on the results of an intensive sampling effort that spanned 60 river miles of the Se-impaired Lower Gunnison River Basin (Colorado) in 2015 and 2016. Concentrations of Se and Hg in green algae (n=12), macroinvertebrates (n=39), and Speckled Dace (n=78) ranged from 1.59 – 12.9 and 0.009 – 0.168 ppm dw, respectively, and increased with trophic level. Mean Se:Hg molar ratios in macroinvertebrates were 286 ± 56 and 237± 117 in Speckled Dace. Initial results provide associative evidence that Hg concentrations in consumers are not inversely related to Se

concentrations in their diet. Mechanisms and implications of potential Se-Hg interactions for Hg bioaccumulation and biomagnification will be discussed.

**2.11.T-05 Measuring In-Situ Surface Water Methylmercury Concentrations Using a Novel Equilibrium-Based Passive Sampler**

***Jada Damond***[1], *Spencer Washburn*[2], *Scott C. Brooks*[2], *Cynthia Gilmour*[3] *and Upal Ghosh*[1], *(1) University of Maryland, Baltimore County, (2) Oak Ridge National Laboratory, (3) Smithsonian Environmental Research Center*

Mercury is a global pollutant that is transformed in aquatic environments into the more toxic and bioaccumulative methylmercury (MeHg). Humans exposed to elevated levels of MeHg, primarily through the consumption of contaminated fish, are at risk for adverse health effects. Aqueous measurements of MeHg are a good predictor of bioavailability to aquatic organisms and are crucial for adequate site risk assessment and remedial practice; however, because MeHg undergoes rapid demethylation, leading to high temporal variability in concentration, and because concentrations are often below instrument detection limits (pM at uncontaminated sites) quantifying MeHg through traditional grab sampling is difficult. Passive sampling can provide time-integrated measurements and improved detection limits. This work presents results of the first surface water field deployment of a novel equilibrium-based passive sampler for MeHg composed of activated carbon suspended in agarose gel (ag+AC). Previous tests of these samplers in laboratory studies using environmentally relevant matrices showed that they provided robust estimates of aqueous MeHg concentrations. Samplers were deployed to measure sub-ng/L MeHg concentrations in surface waters at two locations in East Fork Poplar Creek (EFPC) in Oak Ridge, TN. EFPC is impacted by extensive mercury contamination arising from lithium isotope separation at the Oak Ridge Y-12 security complex in the mid-20$^{th}$ century. The ag+AC passive samplers were deployed for 7 and 14 days, after which samplers and corresponding water grab samples were analyzed for MeHg. Aqueous predictions using the measured MeHg concentration in the sampler and a previously determined partitioning coefficient were within a factor of 1.6 of the direct water measurements at both timepoints. This work demonstrates that these ag+AC samplers are a useful tool to obtain reliable, time-integrative *in-situ* measurements of aqueous MeHg concentrations in environmental deployments. Future work involves simultaneous *in situ* passive sampling of sediment porewater and surface waters, which are useful for measuring flux across the sediment-water interface.

**2.11.T-04 Discussion 1 of 2 - Quantifying the Fate and Effects of Metals**

**2.11.T-06 Re-Thinking the Kinetics of Metals in Sediment Passive Samplers Using Reverse Tracers**

***Florent Risacher***[1], *Jason M. Conder*[1], *Brent Pautler*[2], *Alexander Sweet*[2] *and William Jackson*[3], *(1) Geosyntec Consultants, Inc., Canada, (2) SiREM, Canada, (3) Texas Tech University*

Measuring metal availability in sediment porewater can be a powerful tool for assessing the ecological risk of contaminated sediments, planning remedial actions, or measuring remedial success. Passive sampling using diffusion-based dialysis samplers (“peepers”) are widely used for measuring the availability of metal in sediment porewater. Peepers function by allowing the sampled water to equilibrate with lab-provided water contained in an isolated compartment via passive diffusion through a semi-permeable membrane. After an equilibration period, the peeper is retrieved, and the peeper solution is analyzed for metals (e.g., Zinc, Mercury) and reported as a concentration in water that can be compared to a criteria or used in fate modeling.

It is often assumed that peepers reach complete equilibrium during contact with sediments, with typical exposure times of days to weeks depending on peeper design. However, this assumption may not always be met, leading to a potential underestimation of the concentration of metals in sediments porewater. To address this issue, a reference analyte, or reverse tracer, can be added to the peeper water before exposure to sediments. During exposure, the reverse tracer diffuses out of the peeper into the sediment. After exposure, the concentrations of both tracer and metals are measured. Assuming exponential decay, the reverse tracer data allow to apply a correction factor to metal concentrations that considers the diffusion kinetics.

As part of an Environmental Security Technology Certification Program (ESTCP) project that aims to standardize the use of peepers to measure sediment porewater metal availability, we conducted a series of laboratory experiments in sediments and water with a standard commercially available 15-mL polypropylene peeper (SP15, www.siremlab.com). The work tested the use of reverse tracers (bromide and lithium) to predict equilibrium concentrations of metals in water and in sediment porewater using 1- to 28-day peeper exposure. Results indicated that diffusion kinetics are controlled by the membrane, slowing down the uptake rate of metals by peepers which correspond to lower diffusion coefficients compared to values available in the literature. Bromide and lithium reverse tracers also showed similar diffusion properties, confirming that the kinetics are not controlled by the theorical diffusion coefficients. These results and their implications for measuring metals in porewater will be presented.

**2.11.T-07 Unravelling Iron (III) Bioavailability in Freshwater Samples - Methods and Performance**
***Emiliano Balsamo Crespo***[1]*, Amanda Reichelt-Brushett*[1]*, Ross Smith*[2]*, Andrew L. Rose*[1] *and Graeme Batley*[3]*, (1) Southern Cross University, Australia, (2) Hydrobiology, Australia, (3) CSIRO, Australia*
The toxicity of iron (III) in fresh waters has been detected at concentrations above the iron solubility limit, indicating that this must involve colloidal and particulate forms. Current water quality guideline values for iron (III) in fresh water are based on analytical determinations of filterable or total iron. Such methods are, however, conducive to the underestimation of the colloidal fraction, or the recovery of fractions of low bioavailability from suspended solids (e.g. mineralised iron oxides and oxyhydroxides) naturally abundant in many surface waters. Consequently, there is a need for an analytical method that permits the determination of a bioavailable iron fraction, while avoiding both false negative and false positive results. Ideally a measurement technique is required that can be readily applied by commercial laboratories and field sampling personnel, and integrated into established regulatory schemes. The current study investigated the performance of pH 2 and pH 4 extractions to estimate a bioavailable iron (III) fraction. Synthetic water samples were grouped into four categories according to their iron source: freshly spiked iron, well-mineralised iron, combined mineralised and freshly spiked iron, and a blank group. Because of the possibility of transformation of iron species, the effects of ageing on the iron spike was studied after 1, 3, 7, and 14 days of incubation. The results showed that, the total recoverable, 0.45 µm filtered, and pH 4 acid-soluble fractions neither recovered the added spike, nor discriminated ageing treatments adequately. Contrastingly, the pH 2 extraction showed specificity towards iron phases and ageing groups, particularly within the 0.5- and 2-hour interval. Extraction times above 4 hours and up to 16 hours equally recovered >90% of the spiked iron, regardless of their age. Most importantly, the pH 2 extraction recovered less than 1% of the well-mineralised iron phase. This study shows that a pH 2 dilute-acid extraction is a suitable method to describe a bioavailable iron fraction avoiding false negative and false positive results. The advantages of an extraction time of 0.5 versus 4-16 hours will be discussed.

**2.11.T-08 Discussion 2 of 2 - Quantifying the Fate and Effects of Metals**

**2.11.P Quantifying the Fate and Effects of Metals: Balancing Complexity with Practicality**

**2.11.P-Tu054 Spatial and Temporal Distribution of Heavy Metals in Apalachicola Bay**
***Adebayo S. Solanke***[1]*, Michael Martinez-Colon*[1]*, Olugenga Fajemila*[2] *and Charles Jagoe*[1]*, (1) Florida A&M University, (2) Osun State University, Nigeria*
Estuaries are transitional environments between marine and freshwater systems. Unfortunately, these ecotones function as "natural reservoirs" of heavy metals, pesticides, microplastics, and other polluting compounds. Apalachicola Bay in Florida is known for high yields and commerce of its eastern oyster (*Crassostrea virginica*). However, there has been a decline in oyster harvesting, and Florida fishery regulators approved a

moratorium (2020–2025) to allow the recovery of oysters. It is unclear what environmental stressors (e.g., nutrients, heavy metals, and pesticides) contributed to their decline. This study analyzed levels of heavy metals in the surface and core sediment samples from the bay. The historical changes in enrichment factors over the past 92 years suggest that Zn-Pb-Cr-Cu-Ni-Cd have "minimal enrichment" while "significant enrichment" is observed for Co and Se. However, the Pollution Load Index (PLI) suggests that the aggregation of all the heavy metals is causing a constant "progressive deterioration" of the estuary, which is indicative that pollution exists. It is essential to highlight that during the 2011 drought, a sharp decrease in concentrations was observed for Co-Pb-Zn (lowest values) coupled with the PLI. Heavy metal distribution heat maps show that the provenance of most metals seems to be from local sources within, as evidenced by two depocenters towards the center of the bay. In contrast, the highest concentrations for Cd, Ti, and Se found between seven km upstream and the Apalachicola River mouth strongly suggest that these heavy metals have a direct "watershed" provenance.

**2.11.P-Tu055 Peeping Into Deoxygenation: Experiments to Determine Effects of Oxygen on Peeper Samplers**

***Alex Sweett***[1]*, Jason M. Conder*[2]*, Florent Risacher*[2]*, Brent Pautler*[1]*, Haley Schneider*[2] *and William Jackson*[3]*, (1) SiREM, Canada, (2) Geosyntec Consultants, Inc., Canada, (3) Texas Tech University*

Dialysis passive samplers (i.e. peepers) have been extensively studied and are a simple design that allows an ultrapure water solution to come to equilibrium with the surrounding sediment porewater via diffusion through a semi-permeable membrane. Upon retrieval, the water within the sampler is subjected to laboratory analysis for the target analytes by a standardized method. To date peepers have been produced and used under a wide variety of protocols and procedures without standardized methods or documentation. The benefits of a passive sampler, especially for metals analysis, is the sediment remains undisturbed and no oxygen is added to the sample by doing so, thus minimally impacting the redox sensitive analytes however, there is still a lot of uncertainty with regards to the impact oxygen has on these samplers. Deoxygenation is a costly addition in terms of the prep work it requires to keep peepers deoxygenated prior to field deployment and during processing post retrieval. We set out to conduct multiple experiments to determine the impact oxygen as at various stages during the production, deployment, retrieval and processing by designing tests to measure the potential critical points for oxygen contamination during pre and post deployment phases. These experiments included looking into the necessity for deoxygenation pre-deployment, the shelf life of peeper samplers, and samples (post retrieval). Lab scale study results show that oxygenation contamination occurs as quickly as hypothesized, however, the need for deoxygenation of the peeper solution may not be necessary. Our studies show that, when left in air, peepers only stay deoxygenated for less than 30 minutes. However, the metals data correlated well with samplers that were kept deoxygenated during deployment 28000 µg/L, compared to the oxygenated sampler 26000 µg/L, showing less than ten percent difference. These experiments answered a lot of the questions within literature about deoxygenation of peepers.

**2.11.P-Tu056 Characterization of binding and/or interaction between Hg(II) and AgNPs in aquatic environments**

***Peter Olusakin Oladoye****, Guangliang Liu and Yong Cai, Florida International University*

One of the global pollutants that is reckoned with is mercury (Hg). It is present in environmental media and can be released naturally and anthropogenically through different sources. Atmospheric Hg ($Hg^o$ and Hg(II)) can be deposited into natural water by wash-out or rain-out whereby Hg primarily exists as Hg(II) and transformed to other Hg species. Photochemical redox reaction is a major process that leads to transformation of Hg species from one form to another via oxidation or reduction to generate organo-mercury and/or dissolved gaseous Hg. Suspended particles in water can bind Hg species to produce particulate Hg which is expected to participate in Hg transformation.

This study focuses on the characterization of the interaction between Hg(II) and AgNPs (20 nm) as a representative of engineered nanoparticles being released into the environment. Findings revealed that the DLS measurements (<$2R_H$>) of Hg-AgNPs suspension in the dark (5 mg in 10 mL of acetate buffer of pH6.87) indicates an increase in the hydrodynamic diameter (about three times) upon interaction with 5 ppm of Hg(II), ascribed to sufficient electrostatic interaction time for AgNPs and Hg(II). However, the <$2R_H$> value of the Hg-AgNPs micro-sized within the first 10 min of reaction and later nano-sized after 60 mins, under the light. The energy from the light may be responsible for rapid Hg(II) and AgNPs interaction due to surface plasmon resonance while the reduced size with time may imply reduction of bound Hg(II) to Hg(0) that is less water-soluble.

Thermodynamic investigations on AgNPs-water distribution of Hg(II) were conducted by using 5 mg of AgNPs and 0-500 ng/L of Hg(II) at 15-35$^{o}$C, using cold vapor atomic fluorescence spectrometry for Hg analysis. The distribution coefficients (are found to increase linearly with temperature (15-35$^{o}$C), from which relationship thermodynamic parameters were obtained. This Hg bound by AgNPs warrants for further investigations on its photochemical reactivity and role in aquatic Hg cycling.

## 2.11.V Quantifying the Fate and Effects of Metals: Balancing Complexity with Practicality

### 2.11.V-01 Mercury in the Deepwater Redfish (Sebastes mentella) from the St. Lawrence Estuary and Gulf: Spatial Distribution and Exposure Implications

***Fella Moualek***[1]*, Dominic Belanger*[2]*, Mathieu Babin*[1]*, Geneviève Parent*[3]*, Dominic E. Poton*[2]*, Marc Amyot*[2]*, Dominique Robert*[1] *and Zhe Lu*[1]*, (1) Institut des Sciences de la Mer de Rimouski, Canada, (2) University of Montreal, Canada, (3) Fisheries and Oceans Canada, Canada*

The fate of mercury in the deep sea (>200m), particularly in Canada's Estuary and Gulf of St. Lawrence (SLEG), is largely unknown. According to the marine life survey conducted by Fisheries and Oceans Canada in 2019, Deepwater redfish (*Sebastes mentella*) accounted for 90% (about 15% in previous years) of the total biomass of everything caught on the seafloor in the St. Lawrence Gulf. The inexplicable rise of redfish may have significant impacts on the food web structure and biogeochemical cycling of metals, including mercury, in this region. In addition, this population increase of redfish may result in a large-scale fishery for this deep-sea species after a more than 25-year moratorium. As a result, it is critical to determine whether redfish consumption is safe for humans in terms of mercury exposure. To this end, this study aimed to (1) investigate the concentrations and spatial patterns of total mercury (THg) and methylmercury (MeHg) in the muscles of redfish from the SLEG; (2) explore the correlations between the levels of these contaminants with biological/environmental variables; and (3) conduct a preliminary human health risk assessment of mercury by redfish consumption. The THg and MeHg concentrations were in the ranges of 7.40 - 737 ng/g and 7.14 -581 ng/g wet weight (ww), respectively. MeHg accounted for 93 ± 8 % (mean ± SD) of THg in the muscle of redfish. The muscle of large (>30 cm) redfish contained significantly more MeHg than that of small redfish (12-29 cm). Small Redfish from the St. Lawrence Estuary, the Anticosti Channel, and the Esquiman Channel had higher MeHg levels than other sampling locations. In contrast, large Redfish from the Laurentian Channel and sites near St. George's Bay in Newfoundland had higher levels of MeHg. THg and MeHg concentrations did not differ significantly between female and male redfish. The positive correlation between $\delta^{15}$N and MeHg indicated that feeding ecology variation is an important factor in driving Hg accumulation in small redfish. For human health risks, the average fish consumers face a low health risk, while excessive consumers, who consume the most contaminated redfish, face a moderate risk. This research establishes a baseline for future monitoring of the fate of THg and MeHg in the SLEG's deep-sea environment.

## 2.12.P Selenium Dynamics, Bioaccumulation Potential and Associated Toxicity in Aquatic Ecosystems

### 2.12.P-Tu058 Selenium Site-Specific Standards for Fishless Reaches Using a Hybrid Approach

***Sarah Skigen-Caird****, Daniel Guth and Steven Canton, GEI Consultants, Inc.*

The U.S. Environmental Protection Agency (EPA) finalized tissue-based selenium (Se) criteria in 2016. In fall of 2021, EPA released (revised) draft technical implementation guidance documents to assist states and dischargers with interpreting and implementing the tissue-based criteria into states' water quality standards and associated discharge permits. However, these documents still lack clarity as it relates to methods for implementation within fishless reaches in areas where naturally occurring Se concentrations persist due to underlying geology. Instances where fish are absent in the upstream extent but persist in the downstream extent, suggests that a hybrid approach to calculation of a protective water column number, when needed, may provide an alternative. The criteria document presents two modeling approaches for derivation of site-specific standards: the mechanistic biodynamic model (MBM) and the bioaccumulation factor (BAF). While both approaches can be used where fish are readily available, and typically result in very similar values when populated with site-specific data, neither can be used for fishless waters unless EPA's default trophic transfer factors (TTFs) are used for the MBM. Therefore, in areas where fish are absent due to a lack of habitat, and Se is present intermittently, we propose use of the MBM through employment of EPA's default TTFs. Additionally, we recommend use of site-specific parameters (e.g., water and periphyton, detritus, or sediment) for calculation of the enrichment factor (EF). The use of site-specific data for calculation of the EF is a critical element within the MBM, accounting for the initial transformation from water to biota. The EF can also be highly variable across sites; therefore, the use of site-specific data is an important consideration if use of default TTFs are proposed. This approach allows for the use of the EPA's relatively conservative default TTFs to be used in combination with a site-specific EF for the back-calculation of a protective water column number for use in discharge permits, should it be needed. We continue to maintain that the BAF is a suitable, and preferred, approach for fish-dominated reaches where tissue is readily available, but this hybrid approach may allow for tackling more challenging fishless reaches where data are limited. A case study from a site in the western U.S. will be used to provide an example of this hybrid approach.

**2.12.P-Tu059 Effects of Dietary Selenium on the Freshwater Amphipod, Hyalella azteca**

***David Harper**, John Besser, Rebecca Dorman and Aïda Farag, U.S. Geological Survey*

Based on reproduction, early life stage development, and survival, fishes are the most sensitive aquatic organisms to chronic toxic effects of selenium (Se). The available chronic Se toxicity data for freshwater invertebrates indicate that invertebrates are less sensitive to chronic Se toxicity than fish. However, the number of suitable toxicity studies with invertebrates was limited (only 3 species), and not all these studies included reproductive endpoints. The U.S. EPA was interested in the development of additional invertebrate toxicity data to confirm that invertebrates are protected by the selenium criterion. To address these limitations, a 28-d feeding study was conducted to compare the sensitivity of Hyalella azteca and Chironomus dilutes fed a diet of a combination of selenized yeast and diatoms (0.27, 12.5, 50 and 200 µg/g Se). To further evaluate the toxicity of Se to the amphipod H. azteca, a 42-d chronic feeding study was conducted. The H. azteca were fed the same diet of selenized yeast and diatoms (0.27, 6.25, 12.5, 25 and 50 µg/g Se) Endpoints for the 28-d exposures were survival and growth. Endpoints for the 42-d exposures were survival, growth, reproduction (offspring/female), and Se tissue concentrations. The 28-d study found that effects of Se on H. azteca were observed at lesser concentrations than C. dilutus. Chronic 42-day exposures with H. azteca produced an EC20 for growth and biomass (end dry weight) of 22 µg/g Se dry weight in the diet. Preliminary results indicate effects on reproduction as low as 3 µg/g. However, the data must be further analyzed due to the novel feeding approach with a yeast/diatom mixture which resulted in control reproduction below test acceptability criteria.

**2.12.P-Tu060 Use of Fish Tissue Concentrations in a Reasonable Potential Analysis Results in the Removal of Selenium Limits**

*Daniel Guth, Jennifer Lynch, Steven Canton and **Sarah Skigen-Caird**, GEI Consultants, Inc.*

The U.S. Environmental Protection Agency (EPA) finalized the tissue-based selenium (Se) criterion in 2016. In fall of 2021, EPA released (revised) draft technical implementation guidance documents to assist states and dischargers with interpreting and implementing the tissue-based criteria into states' water quality standards and associated discharge permits. However, these documents still lack clarity on how a four-part criterion should be used for a reasonable potential analysis. In this presentation, we will discuss the steps taken by a discharger towards the removal of Se limits in their discharge permit. These steps included proposing the 2016 Se criterion as a site-specific standard on their receiving streams (a standard later adopted by the state and approved by EPA). We will also review the groundwork taken to establish existing Se concentrations, in both the water and in fish tissues downstream of the outfall, that ultimately led to a finding of no reasonable potential by the permitting agency and removal of the numeric Se limits from their National Pollutant Discharge Elimination System (NPDES) permit.

**2.12.P-Tu061 Bioaccumulation of Selenium through the food chain: water - Lemna minor - Pomacea paludosa**

***Tham C. Hoang***[1] *and Avais Ahmed*[2]*, (1) Auburn University, (2) Loyola University Chicago*

Selenium (Se) has been used as a fungicide in sugar cane growth in South Florida for many decades. The long-term use of Se in these environments has led to an increase in Se concentrations in the soil. Under the Comprehensive Everglades Restoration Plan, thousands of acres of agricultural lands will be flooded to create wetland habitats and water reservoirs. Flooding lands will result in Se desorption from soils to water and would affect aquatic organisms. Aquatic plants such as *Lemna minor* (duckweed), inhabitants of these systems, can accumulate Se which then can be transferred via predation to higher trophic levels. The objective of this study is to study how Se transfers through different levels of the food chain (water-duckweed –*Pomacea paludosa* (Florida apple snail). Under standard laboratory conditions, duckweed was exposed to three different concentrations of Se (100 µg/L, 500 µg/L, 1,000 µg/L) for 2 weeks. Apple snails were fed with Se contaminated duckweed for 28 days. During the course of the study, apple snails and duckweed were collected at different time intervals for Se analysis. This study found that Se was bioaccumulated from water to duckweed and apple snails. Se concentrations in duckweed increased with Se treatment concentration and ranged from 1 mg/kg dw (control) to 431 mg/kg dw (1,000µg/L treatment). Concentration of Se in the snail soft tissue (food + viscera) increased with increasing Se duckweed concentration and ranged from 0.77 mg/kg dw (control, day 0) to 142 mg/kg dw (1,000µg/L treatment, day 21). In general, Se concentration in apple snails increased in the order of shell, food, and viscera. Se concentration in the snail soft tissue also increased with exposure time. Results of the present study indicate that Se can be bioaccumulated through the food chain. This poses potential exposure to organisms at different tropic levels in aquatic ecosystems.

**2.12.P-Tu062 Selenium: Mercury Interactions and Implications for Toxicity**

***Alexandra Duguay****, Rio Tinto, Canada*

There are two over riding factors that should be consider when reviewing selenium (Se) and mercury (Hg) in aquatic life: (1) Protective effects of selenium against mercury toxicity have been demonstrated in all animal models evaluated (fish, birds, mammals and plants); and (2) both elements in biological tissues can be converted to an insoluble, non-bioavailable form as mercury selenide. Since interactions between Se and Hg and their molar ratios in seafood are essential factors in evaluating risks associated with dietary Hg and Se, considering either element alone is an inadequate approach to assessing risk to a given organism. The data presented in this review show that most fish tissues in the wild contain Se/Hg ratios >1.0. When the molar ratio of Se/Hg is <1.0 in the diet of animals and humans there is evidence that mercury toxicity can be expressed, and that this toxicity is the result of Se deficiency and excess reactive oxygen species. EPA tissue thresholds for selenium toxicity have been derived primarily from studies where the fish were not exposed to Hg, which maximizes the potential for Se toxicity resulting in conservative thresholds. The overall conclusion is that the presence of the selenium reduces the potential for Hg toxicity while at the same time, Hg limits a portion of the Se available for metabolic functions.   The data further suggests that the EPA fish whole-body tissue threshold of 8.5 µg/g is conservative since it is derived without consideration of the interaction of mercury.

**2.12.P-Tu063 Investigating the uptake kinetics of selenite and selenate in natural periphyton representative of lotic systems**

***Esteban Gillio Meina**, Katherine Raes, Kerstin Bluhm, Susari Malala Irugal Bandaralage, Tabata Bagatim, Francisco da Silva, Karsten Liber, Som Niyogi and Markus Hecker, University of Saskatchewan, Canada*
Selenium (Se) is an essential micronutrient for many aquatic organisms, but it can become a contaminant of concern for oviparous vertebrates when present at elevated concentrations due to it's bioacccumulative and teratogenic properties. The initial uptake of inorganic Se species by natural periphyton and microorganisms is considered to be the critical step in accumulation and trophic transfer of Se in aquatic ecosystems. Primary producers convert inorganic Se into organo-selenium compounds, thereby increasing the bioavailability of Se for higher and more sensitive trophic level consumers. Still, little is known about how Se speciation and variations in water quality characteristics influence this initial uptake and accumulation of Se in natural periphyton of lotic systems. In this study, radiolabled Se as selenite ($^{75}$Se(IV)) and selenate ($^{75}$Se(VI)), were used to measure the uptake of Se in natural periphyton communities grown in a cold-water lotic environment. In addition, the influence of sulphate (30-600 mg/L), an ion that competes with selenate for uptake, on $^{75}$Se(VI) accumulation in natural periphyton was studied. Preliminary results suggest that Se(IV) and Se(VI) have different uptake kinetics. Se(IV) exhibited a linear accumulation, whereas Se(VI) accumulation was best described by a quadratic model. Furthermore, when Se(IV) and Se(VI) accumulation were compared,$^{75}$Se(IV) accumulation in periphyton was much faster than that of $^{75}$Se(VI). In addition, a decrease in $^{75}$Se(VI) accumulation in natural periphyton was identified with increasing sulfate concentrations in the test water. Overall, these results suggest a need to further investigate the differences in accumulation between the two Se species and how water chemistry could influence their biokinetics.

### 2.12.P-Tu064 Relationships in Selenium Concentrations Among Fish Tissues: Monitoring and Regulatory Implications

***Claire Detering**[1], Kevin Brix[2], Marko Adzic[3], David K. DeForest[1] and Barry Fulton[4], (1) Windward Environmental LLC, (2) EcoTox LLC, (3) Teck Resources Ltd., (4) Benchmark Environmental LLC*
Several regulatory jurisdictions in North America have adopted fish tissue-based selenium criteria or guidelines. The US Environmental Protection Agency (USEPA), for example, developed recommended selenium criteria of 15.1 mg/kg dry weight (dw) for fish eggs and ripe ovaries, 11.3 mg/kg dw for fish muscle, and 8.5 mg/kg dw for whole-body fish tissue. Because selenium's primary mechanism of toxicity occurs via maternal transfer to ovaries and eggs, which can impair larval development and survival, the Se criterion for fish eggs and ripe ovaries supersedes muscle and whole-body Se criteria. However, it can be logistically challenging to sample eggs or ripe ovaries in the field. In addition, it may be desirable to conduct non-lethal sampling of muscle tissue or to combine resources with other fish monitoring programs that may focus on collection of muscle or whole-body tissue in support of human health assessments. Relationships for estimating egg or ripe ovary Se concentrations from muscle or whole-body Se concentrations is desirable. In this study, we first developed a database of selenium concentrations that could be paired in two or more fish tissues (eggs/ripe ovaries, muscle, and whole-body). When available, we also compiled information on the reproductive status of the fish sampled. Relationships in selenium concentrations among fish tissues were evaluated for more than 50 freshwater fish species using linear regression. Several fish species exhibited an inverse relationship between ovary selenium concentrations and spawning status (i.e., ovary selenium concentrations were lower in reproductively mature fish near spawning). These results emphasize the importance in developing relationships between muscle/whole-body and ovary selenium concentrations based on ovaries that are ripe. We supported this analysis by evaluating variability in ovary selenium concentrations as a function of spawning strategy (i.e., synchronous and asynchronous spawners). Asynchronous spawners may contain ovary tissue with eggs at various levels of development, which can preclude confident sampling of ripe ovaries. For these fish, muscle or whole-body selenium concentrations may be a more reliable measure of selenium exposure and assessments of potential effects. The selenium relationships among fish tissues developed in this study may be a helpful resource as states continue to adopt and implement the USEPA's recommended fish tissue selenium criteria.

## 2.13 Understanding the Ecological Effects of Nanoplastics in Aquatic Environments

**2.13.T-01 Introductory Remarks - Understanding the Ecological Effects of Nanoplastics in Aquatic Environments**

***Kay T. Ho**[1], Susan B. Kane Driscoll[2] and Robert M. Burgess[1], (1 )U.S. Environmental Protection Agency, (2) Exponent*

**2.13.T-02 Assessing Effects of Nano- and Microplastics in Aquatic Environments**

***Richard Zepp**[1], Brad Acrey[1], Wendel Wohlleben[2], Endalkachew Sahle-Demessie[1] and Seth McWhorter[3], (1) U.S. Environmental Protection Agency, (2) BASF SE, Germany, (3) Oak Ridge Associated Universities*

Microplastics (MPs) (defined as plastic particles < 5 mm in size) and microfibers are widely distributed in terrestrial and aquatic ecosystems. The plastics are often combined in composites with nanomaterials such as carbon nanotubes or graphene oxide that maximize desirable properties such as strength, conductivity, and antibacterial activity. Assuming current trends in production and no improvements in waste management, releases of MPs into the environment may grow to 90 metric tons per year by 2030. The ecological risks of MPs are closely linked to their exposure concentrations in aquatic environments. One significant source of MPs involves combined UV-initiated and mechanical fragmentation of these particles into smaller and smaller sizes. The interaction between UV light and plastics may also be an important pathway for the eventual removal of plastic particles from the environment. For example, exposure to UV radiation on the ocean surface can transform common plastics such as polystyrene into $CO_2$ and dissolved organic carbon. Across ecosystems, many of the effects of microplastics are influenced by their physical characteristics, including particle size and shape. Because exposure to UV radiation affects these characteristics, it may play a key role in regulating potential toxicity and ecological impacts of microplastics. Here we report our recent efforts to use the ISO-4892-2 protocol for testing plastics degradation and to develop methods for evaluating the fragmentation of macroplastics and MP by weathering into "secondary", smaller MPs with concurrent release of associated chemicals, including carbon nanotubes and graphene oxide. Our studies provide new information on developing methods for microplastic isolation and analysis that minimize interferences from dissolved organic matter.

**2.13.T-03 Differences in the Uptake and Accumulation of Nanoplastics Between Oral and Waterborne Exposures Using Larval Zebrafish (Danio rerio)**

***Jordan Avery Pitt**, Neel Aluru and Mark Hahn, Biology, Woods Hole Oceanographic Institution*

Microplastics and nanoplastics are found in marine biota across a wide range of trophic levels and environments. While a large portion of the information about plastic exposure comes from gastrointestinal (GI) data, the relevance of an oral exposure pathway compared with other potential pathways of exposure (e.g. dermal, respiratory) for accumulation of particles in other tissues is poorly understood. To address this gap in knowledge, larval zebrafish (7 days post fertilization) were exposed to nanoplastics (50 nm and 500 nm fluorescent polystyrene particles) through either oral gavage (1.2 µg/larva) or a waterborne exposure (1 mg/L). The oral gavage exposure limited potential accumulation to one uptake path while the waterborne exposure could lead to a combination of oral, dermal, and respiratory (gills) uptake. The exposures were done using the unpigmented mitfa transgenic line to allow for imaging at later developmental time points. Individual larvae were monitored by confocal microscopy for 48 hours post exposure (hpe) to assess the migration and elimination of plastics from the GI tract and into other tissues. Larvae exposed to nanoplastics (50 nm and 500 nm) by oral gavage were able to eliminate the plastics by 48 hpe. Migration of particles from the GI tract into other tissues was not detected. In contrast, larvae exposed to nanoplastics via an aqueous suspension displayed fluorescence in tissues outside of the gut. The larvae exposed to the 50 nm particles were not able to fully eliminate the particles by 48 hpe, and showed accumulation that appeared to be associated with neuromasts, as suggested by experiments using the zebrafish transgenic line Tg(*tnfa*:eGFP), which labels neuromasts. The larvae exposed to the 500 nm waterborne particles displayed particle aggregations throughout the vasculature shortly after exposure. This finding is being further investigated using the transgenic zebrafish line Tg(*kdrl*:dsRed), which labels the zebrafish vasculature. For both sizes of plastic particles, the accumulation

outside of the GI tract was largely eliminated by 24 hpe. This work indicates that oral exposures may be less relevant than other exposure pathways in determining the bioaccumulation potential of plastics in some organisms. The short residence time in the body post exposure potentially indicates the low bioaccumulation potential of some nanoplastics. [Supported by NOAA Sea Grant, an NSF Graduate Fellowship, and the WHOI Academic Programs Office.]

**2.13.T-04 Effects of Micro- and Nano-Scale Plastic Exposures in Blue Mussels, Mytilus edulis**
***Bushra Khan***[1]*, Zahra Zahra*[2]*, Bridget Holohan*[3]*, J. Evan Ward*[3]*, Adeyemi Adeleye*[2]*, Troy Langknecht*[1]*, Robert M. Burgess*[1] *and Kay T. Ho*[1]*, (1) U.S. Environmental Protection Agency, (2) University of California, Irvine, (3) University of Connecticut*
Plastic production and usage have increased dramatically over the last several decades. Due to their durability, inadequate waste management and resistance to degradation, plastics tend to accumulate in landfills and aquatic habitats. Upon entering marine ecosystems, these contaminants can further break into micro- and nano-scale particles which can adversely affect resident biota. Due to their surface properties and small size, plastic particles can lead to cellular damage in marine organisms. Micro- and nano-scale plastics (MP and NP, respectively) can be internalized by suspension-feeding bivalves such as the blue mussel, *Mytilus edulis*. Here, we present results from a 14-day static renewal study with blue mussels exposed to micro- and nano-scale polystyrene (3 µm and 50 nm, respectively) at an effective concentration of 0.1 mg/L/hr. The high concentration was selected so that biomarker responses could be detected and differences between MP and NP effects could be quantified. Effects in gill and digestive gland tissues were evaluated using biochemical and metabolomic approaches. Biochemical assays included assessments of the ratio of oxidized and reduced glutathione, lipid peroxidation and total protein levels. Additionally, significantly impacted metabolites and affected pathways were identified using metabolomic analyses. Our results show the key effects of MP and NP exposures are on cellular antioxidant machinery and redox status. Energy metabolism was also impacted by exposures, representing shifts in energy demands of detoxification processes. Metabolites related to immune function and inflammatory pathways were affected, likely due to particle-induced cellular injury. Overall, digestive gland tissues of NP-exposed mussels showed the most changes in cellular machinery. These results indicate polystyrene particle exposures can result in the generation of reactive oxygen species (ROS) that are involved in several antioxidant and inflammatory signaling cascades. Further investigations are warranted into the effects of MP and NP exposures, at lower environmentally relevant concentrations, on the interactions between ROS, antioxidant responses and immune pathways. Molecular and cellular interactions between signaling cascades can help us understand changes in energy homeostasis affecting adverse outcomes at individual levels.

**2.13.T-05 Effects of Polystyrene Nanoplastic on Benthic Microbial Communities**
***Marissa Giroux****, Jay R. Reichman, Troy Langknecht, Bonnie M. Smith, Robert M. Burgess and Kay T. Ho, U.S. Environmental Protection Agency*
Plastic particles are found in aquatic systems worldwide, and the effects of plastics on ecosystem health are of growing concern. Macro- and microplastics fragment into nanoplastic particles (<1 µm) in the environment, often accumulating in sediments. Marine sediments act as a sink for many contaminants including plastic particles and are also rich habitats for microbial communities. Microbes are vital for ecosystem maintenance as foundational components of food webs and contribute to biogeochemical processes such as carbon and nutrient cycling. However, some contaminants adversely affect microbial communities and indirectly alter environmental conditions, thus changing the balance of microbial communities. Macro- and microplastics can be substrates for microbial growth, but less is known about the interaction of nanoplastics and microbes. Environmental DNA metabarcoding allows for rapid and comprehensive detection of microbial communities via high-throughput sequencing to assess community structure and function. The objective of this study was to use a 16S rRNA metabarcoding approach to investigate the effects of polystyrene nanoparticles on microbial community diversity and structure. Mesocosms were collected from the Narrow River estuary in Rhode Island (USA) and exposed to seawater-weathered 900 nm diameter nanoplastic spheres at concentrations of 0, 0.1, 1,

10, or 100 mg/kg dry weight amended to a reference sediment for two weeks. Following exposure, DNA was extracted from the top 1 cm sediment layer, 16S rRNA gene marker was PCR-amplified, and amplicons were sequenced on an Illumina MiSeq. A dose-dependent decreasing trend in α-diversity was observed. Additionally, the abundance of anaerobic, sulfur-reducing bacteria increased in higher NP treatments compared to lower treatments. This showcases a change in microbial community structure in response to increasing NP exposure, possibly due to polystyrene as a new source of organic-based material within the mesocosms. These results, and the findings of previous studies evaluating nanoplastic impacts to eukaryotic communities (i.e., meiobenthos), contribute to the understanding of plastic particles directly and indirectly affecting environmental conditions leading to community-level impacts.

**2.13.T-06 Sorption and Quantification of PAHs in the Lavaca Bay using Environmentally Weathered Microplastics.**

***Oluniyi O. Fadare****, Nigel Lascelles and Jeremy L. Conkle, Texas A&M University, Corpus Christi*

The mechanisms of microplastics (MPs) acting as vectors for other contaminants in aquatic ecosystems are complex and not fully understood. Depending on polymer type, weathering impact as well as corona formation, MP properties are significantly influenced in the aquatic environment. This ongoing field study conducted in the Lavaca Bay system (Texas), which has a Superfund Site with mercury and polycyclic aromatic hydrocarbon (PAH) pollution, is designed to improve our understanding of the interactions of MPs and other aquatic contaminants in the environment. We investigated the sorption of PAHs to weathered MP extracted from wrack line debris comprised of mostly particulate organic matter (POM) in Lavaca Bay. Moreover, the abundance, polymer types, and morphological characteristics were determined. From our preliminary results, microplastics accounted for 2.62-21.3% (14.81 ± 7.13) of the wrack line debris mass. From these 1 g samples, MP mass ranged from 60.15 ± 29.58 6 mg $g^{-1}$ to 196.6 ± 15.71 mg $g^{-1}$. All 16 target PAHs were detected, with fluoranthene having the highest average concentration of 6,092 ± 268 ng $g^{-1}$. Total PAH concentration was 28,786 ± 1220 ng $g^{-1}$. Data collection is ongoing. The data generated will further our knowledge of microplastic behavior in the aquatic and coastal systems and its impacts on environmental and human health.

**2.13.T-08 Key Challenges and Limitations to Characterizing and Quantifying Environmental and Human Health Risks to Micro- and Nanoplastic Particles: Reference Materials Urgently Required**

***Todd Gouin***[1]*, John Norman[2], Steven Black[3] and Leah West[3], (1) TG Environmental Research, United Kingdom, (2) American Chemistry Council, (3) ICF*

Micro- and nanoplastic particle (MNP) research is at a nascent stage, with numerous studies observing a need to adopt robust quality assurance / quality control (QA/QC) practices regarding sample collection, analysis and effects testing. Good QA/QC is needed to support the reliability and relevance of data generated, which further supports comparability across studies and which further strengthens the ability to perform a risk assessment. It is generally understood that an important element of QA/QC protocol relates to a demonstrated understanding of the characteristics of the stressor under investigation. The development and application of sampling and analytical methods, for instance, relies on the use of analytical standards, which are used to quantify the efficacy of the sampling and analytical method, such as in the reporting of recovery efficiencies or in the use of quantifying calibration curves. At present, there are no readily available standardized MNP reference materials or methods. Consequently, it is difficult to ascertain the environmental and human health risks from existing studies because the exposure data may be of varying quality and reproducibility. The lack of availability to standard reference materials represents an important barrier towards strengthening the quality of MNP research, and are thus urgently needed. In an effort to address this urgent research need, a multi-stakeholder workshop was held during May 2022 in Atlanta, aimed at exploring opportunities to support the generation of a suite of environmentally relevant standard reference MNP materials for use to support the validation of sampling, preparation, and analytical protocols. MNP reference materials would encompass different resins, morphologies, and sizes to represent in some degree the particle variability present in the environment. Standard materials would serve a variety of needs, but would be particularly valuable in supporting the adopting of good QA/QC practices for both environmental monitoring and effects testing, thus helping to strengthen the quality and reliability of data to support risk-based decisions. This presentation summarizes key output from the workshop, including a summary of the various approaches currently used to generate and weather MNP, their strengths and weaknesses and recommendations regarding best practices for use of MNP reference materials for supporting both analytical method development and effects testing.

## 2.13.P Understanding the Ecological Effects of Nanoplastics in Aquatic Environments

### 2.13.P-We023 Using Weighted-Averaging to Calibrate Stressor-Specific Genus Sensitivity Values for Assessing Causes of Stream Impairments

***Michael Bruce Griffith****, U.S. Environmental Protection Agency*

Effective water quality management is based on scientific evidence or demonstrated associations between at least two pieces of information: a stressor and a response. A large, combined data-set of USEPA and state macroinvertebrate and environmental data was split into a larger calibration data-set and a smaller test data-set. Environmental variables that are measures of stressors were selected for analysis that were uncorrelated and usually had several thousand site observations. Using weighted-averaging with the calibration set-set, stressor-specific genus sensitivity values were calibrated for the selected water chemistry [$\log_{10}$ (X+1) transformed, except for pH] or physical habitat variables. Rather than using these stressor-specific genus sensitivity values to directly infer stressor values, we used these values to classify genera into four sensitivity classes along each stressor gradient and calculate a metric, the Sensitive Genus Ratio = Number of Genera in the Two Most Sensitive Classes / Total Number of Genera, using the test data-set. By using this ratio, the sites could be separated into intervals where the mean Sensitive Genera Ratio was statistically different between intervals for many of the tested environmental variables, including some metals, ions, nutrients, pH, and mean substrate diameter. However, there were weaknesses. Limited observations of an environmental variable in the data-sets limited the number of genera for which a tolerance value could be calculated. Also, when the tails of the distribution of an environmental variable in the data-set were small, the tails were often not statistically different compared to the center of the distribution.

### 2.13.P-We024 Synthesis and Characterization of Microsize Polyisobutylene and toxicological evaluation on the development of Zebrafish (Danio rerio)

***Abass Toba Anifowoshe****, Victor Ayobami Ajisafe, Ashok M Raichur and Upendra Nongthomba, Indian Institute of Science, Bangalore, India*

Polyisobutylene (PIB) is an elastomeric macromolecule that exhibits superior gas and moisture imperviousness, high damping, and chemical and oxidative stability. It is widely utilized in various commercial applications, such as tire inner liners and tubes, sealants, adhesives, condenser caps, pharmaceutical stoppers, and chewing gums, due to its thermal stability, high flexibility at ambient temperature, and ability to pass gases. Limited studies have shown the degradation of PIB in the aquatic environment; however, its microsize toxicity effect has not been studied. In this present study, we synthesized and characterized the microsize PIB and evaluated the toxicity effects and accumulation of the PIB in the zebrafish model. Zebrafish offers many advantages as a research model, including rapid development, optical transparency, a large number of offspring, and an excellent vertebrate model for toxicological research. Using the solvent evaporation method, we synthesized pristine and fluorescence PIB-microplastic (MP) with particle sizes of < 2-10 μm. The FTIR characterization tests showed that the samples have notable peaks at 1366 and 1388 wavenumber ($cm^{-1}$) and zeta potential of approximately -60 mV, indicating the inherent stability of the suspensions. Zebrafish larvae exposed to various concentrations of PIB-MP showed reduced swimming, delayed hatching, increased ROS, and changes in mRNA levels of genes (*mnsod, cu/znsod, gsr,* and *gstp1*) encoding antioxidant proteins, and increased mortality. The PIB-MP was accumulated in the larvae and adult fish gut within 7 to 14 days, respectively. The present study would help in understanding how PIB-MP enters its host and causes pathophysiological defects, which would provide valuable insights for the health hazards evaluation of PIB-MP in the river and future river water treatment strategies.

**2.13.P-We025 Microplastic Accumulation in Aquatic Organisms in Saigon-Dongnai Rivers: A Comparison of Hard Clam, Meretrix Lyrata, and Giant River Prawn, Macrobrachium Rosenbergii**
***Bao-Son Trinh***, *Vietnam National University of Ho Chi Minh City*
Our recent study showed that most of microplastic debris in Sai Gon-Dong Nai rivers are polyethylene (PE). Here we report the accumulation of fluorescent green PE microbead (1.025 g/cm$^3$, dia. 63-75 µm, $5.81\times10^6$ beads/g) in the two typical Sai Gon-Dong Nai aquatic organisms of hard clam (*Meretrix lyrata*) and giant river prawn (*Macrobrachium rosenbergii*). Juvenile clams (6-month old, L×W×H = 7×6×4 mm, 2 g/clam) were exposed in PE suspensions of 0 (control), 1, 10, 20, and 100 mg/L over the periods of 0, 6, 12, 24, and 48 h (5 clams/glass bottle and 3 replicates). Post larvae giant river prawn (12-day old, 10 mm, $7.5\times10^{-3}$ g/prawn) were exposed in the similar PE suspensions of 0, 5, 50, and 100 mg/L over 0, 12, 24, and 48 h (1 prawn/glass bottle and 5 replicates). Results from the treatments with 100 mg/L showed that: after 12-h exposure, significant numbers of the microbeads were accumulated in clam, but not in prawn, with the values of 588 ± 95 and 1 ± 1 beads/organism, respectively; after 24-h exposure, the accumulated PE beads in the both organisms significantly increased, however the beads in clam are higher than the one in prawn, with the values of 626 ± 164 and 14 ± 6 beads/organism, respectively; and finally, after 48-h exposure, the accumulated PE beads significantly decreased, and the beads in clam are still higher than the one in prawn, with the values of 298 ± 9 and 0 ± 0 bead/organism, respectively. It is found that microplastics could be accumulated in both clam and prawn during the first 24-h exposure, however, microplastics could be excreted or regurgitated over 48-h exposure.

**2.13.P-We026 Evaluation of Pyrolysis-Gas Chromatography/Mass Spectrometry Library Matching Programs for Micro- and Nanoplastic Analysis**
***Jennifer L. Gundersen***, *Robert M. Burgess and Kay T. Ho, U.S. Environmental Protection Agency*
As concern about the impact of petroleum-based microplastics and nanoplastics (MNP) grows, numerous analytical methods for MNP in environmental matrices are being developed. To help standardize method development among researchers, Hawaii Pacific University (HPU) assembled and distributed a kit of 22 polymers commonly encountered as MNP. Pyrolysis-gc/ms (pyr-gc/ms) shows promise as a method to both identify and quantify MNP in environmental samples by producing pyrolyzates with distinctive and reproducible mass spectra. Pyr-gc/ms mass spectral libraries are being developed commercially and by individuals for identification of MNP. In this study, the polymers from the HPU kit were analyzed by pyr-gc/ms and evaluated using the commercial pyrolyzate libraries as well as a user-developed library. Pyrolysis conditions affected the pyrolyzates formed and thus the quality of the identification from established libraries. For user-defined libraries, it is critical that pyrolysis and chromatographic conditions be identical in sample and library analyses.

**2.13.P-We027 Detection of plastic debris and microplastics in sea turtles from Japanese coastal waters and estimation of their exposure risk**
***Haruhiko Nakata***[1], *Hiroto Yamada*[1], *Koshiro Eto*[2], *Shinsuke Tanabe*[3] *and Tatsuya Kunisue*[3], *(1) Kumamoto University, Japan, (2) Kitakyushu Museum of Natural History and Human History, Japan, (3) Ehime University, Japan*
In this study, 54 digestive tract contents of green (*n*=31), loggerhead (*n*=11), hawksbill (*n*=11) and leatherback (*n*=1) turtles collected from Japanese coastal waters during the 1980s and 2021 were analyzed for plastic debris and microplastic (MP). In addition, 6 plastic debris found in the digestive tracts were extracted by dichloromethane and the solution was injected into GC-MS for determination of organic plastic additives in samples.

As the results, damaged eyeglass frame and lighter were found in digestive tracts of a hawksbill collected around the Kyushu coast, western Japan in the 1980s. Several fragments of fishing floats and plastic bags were also found in loggerhead turtles from the coast of Shikoku in 1990 and 2006, respectively. These indicate that domestic turtles in Japan have been exposed to plastic debris for more than 30 years, and they could give adverse effects in marine wildlife.

MPs were identified in the digestive tract contents of sea turtles with detection frequencies of 94%, 91% and 55% for green turtles, loggerhead turtles and hawksbills, respectively. Polyester (PES) was the most common polymer (37% of the total), followed by polyvinyl chloride (PVC; 16%) and epoxy resin (EP; 11%). Plastic fiber was dominant in green and loggerhead turtles, and the colors of MP were mostly blue and black, both accounting for about 70% of the total. The mean abundance of MP was significantly higher in loggerhead turtles (35 items/individual) than in green turtles and hawksbills. These values were apparently higher than those in sea turtles in Europe and Australia, and were comparable to those of sea turtles off the coast of Korea. This indicates that MP pollution of sea turtles in the East Asian coast may be more serious than in other regions.

As for additive analysis, benzotriazole UV stabilizers including UV-326 and UV-327 and a representative phthalate ester plasticizer, DEHP, were detected in plastic debris from sea turtles. It is known that UV-327 is persistent and highly accumulated in the environment, and it has added to the list of substances subject to authorization under the REACH regulation. While the production and usage volumes of these chemicals are considered to decrease recently, they are still contained at high concentrations in plastic debris and exposed to wildlife. The bioaccumulation properties of harmful plastic additives on marine organisms, including sea turtles, should be investigated.

**2.13.P-We028 Acute and Chronic Toxicities of Polyethylene Microspheres on Aquatic Organisms Exposed to Plankenburg River Water Samples**

***Komlan Apetogbor,*** *Omoniyi Kolawole Pereao and Beatrice Opeolu, Cape Peninsula University of Technology, South Africa*

Ecological effects of microplastics (MPs) in freshwater systems is a global concern. The ubiquity of microplastics coupled with its adverse impact on the ecological system requires an understanding of the associated risks. The assessment of the ecological risk of microplastics in the Plankenburg River water samples was assessed. Test organisms were exposed to polyethylene in the laboratory and endpoints measured. The three test organisms used were *Daphnia magna, Raphidocelis subcapitata and Tetrahymena. thermophila*. Primary microplastics, polyethylene microspheres (40-48 µm) were used in the experiment. Class III was obtained from site 1 to site 3 (Acute hazard) and the percentage effect (PE) was within 50% and 100% at the different sites in one test (*Tetrahymena. thermophila* growth inhibition assay). However, no other tests showed toxic effects. Anthropogenic activities in the vicinity of the sampling sites were major drivers of toxicity in the Plankenburg River.

**2.13.P-We029 Microplastics Occurrence and Ecological Risks in Wastewater Treatment Plant Effluent, Cape Town, South Africa**

***Omoniyi Kolawole Pereao*** *and Beatrice Opeolu, Cape Peninsula University of Technology, South Africa*

Wastewater treatment plants (WWTPs) release complex combinations of industrial and domestic effluents into aquatic system. WWTP are an important point source of both primary and secondary microplastics (MPs) from domestic waste  that contain microbeads from personal care products and microfibers from the laundry into streams. The occurrence of microplastics was determined in the WWTP effluent and in the discharge river, which flowed through different land-use practices. Physicochemical indicators of water quality were measured to determine effluent quality relative to regulatory limits. The potential risk of WWTPs discharge into waterbodies was evaluated using toxicity bioassays. A battery tests using a primary producer - *Pseudokirchneriella subcapitata*, a consumer -*Daphnia magna* and a decomposer -*Tetrahymena thermophile* were conducted. Physicochemical parameters' values were all within the regulatory limits. The respective values (ranges) for chemical oxygen demand (COD), total dissolved solids (TDS) and electrical conductivity (EC) were 37 – 597.33 mg/L, 623 – 813 mg/L and 0.91 – 1.218 µS/m. The effluent samples were classified as Class II (slight acute toxicity). The occurrence of MPs in the effluent may contribute to the MP load of the receiving waterbodies.

**2.13.P-We030 Occurrence and Toxicity Studies of Microplastics in Effluent Samples of a Wastewater Treatment Plant**

***Sihle Mlonyeni**, Omoniyi Kolawole Pereao and Beatrice Opeolu, Cape Peninsula University of Technology, South Africa*

Wastewater treatment plants (WWTP) are designed to safely dispose wastewater that has been generated during water use, by reducing or removing contaminants that may impact human health and the environment. However, due to population growth, the technologies used are unable to handle the increasing loads received by WWTPs. Emerging contaminants such as microplastics (MPs) further contribute to the deteriorating quality of the WWTPs effluent discharged into rivers. This study assessed the quality of the WWTP's effluent using *Daphnia Magna, Raphidocelis subcapitata* and *Tetrahymena thermophila* as tests species. The occurrence of MPs was determined in the influent and effluent to assess the WWTP's MP removal efficiency. The MPs in water and sediment samples were extracted, digested, and identified using microscopy and Fourier-transform infrared spectroscopy (FTIR). The ecotoxicity classifications showed a very high acute toxicity (class V) for the autumn season, while spring showed an acute toxicity (class III). The most prominent MP forms found in the effluent samples were fibres, with the most common colours being black/grey and 55% of MPs analysed using FTIR-ATR were polyether urethane. The findings of this study showed that dilution of the effluent reduced toxic effects exerted on test organisms. However, design of new technologies that can remove MPs and/or upgrade of WWTPs is now required for water sustainability.

**2.13.P-We031 Screening and Prioritization of Nano- and Microplastic Particle Toxicity Studies for Evaluating Human Health Risks – Development and Application of a Toxicity Study Assessment Tool**

***Todd Gouin**[1], Robert Ellis-Hutchings[2], Leah Thornton Hampton[3], Christine L. Lemieux[4] and Stephanie Wright[5], (1) TG Environmental Research, United Kingdom, (2) The Dow Chemical Company, (3) Southern California Coastal Water Research Project (SCCWRP), (4) Health Canada, Canada, (5) Imperial College London, United Kingdom*

Concern regarding the human health implications that exposure to nano- and microplastic particles (NMPs) potentially represents is increasing. While there have been several years of research reporting on the ecotoxicological effects of NMPs, human health toxicology studies have only recently emerged. The available human health hazard data are thus limited, with potential concern regarding the relevance and reliability for understanding the potential human health implications. In this study we develop and apply a NMP toxicity screening assessment tool (NMP-TSAT) for evaluating human health effects studies against a suite of quality assurance and quality control (QA/QC) criteria for both in vivo and in vitro studies. A total of 74 studies representing either inhalation or oral exposure pathways were identified and evaluated. It is observed that the majority of studies evaluated using the NMP-TSAT have been performed on monodisperse particles, predominately spheres (≈60%), consisting of polystyrene (≈46%). The majority of studies have tested particles < 5 µm, with a minimal particle size of 10 nm and a maximum particle size of about 200 µm. The total assessment score (TAS) possible for in vivo studies is 52, whereas for in vitro studies it is 46, which is based on receiving a maximum score of 2 against 26 and 23 criteria, respectively. The evaluated TAS ranged from between 12 and 44 and 16–34, for in vivo and in vitro studies, respectively. Given the challenges associated with prioritizing studies based on ranking them according to their TAS we propose a Tiered approach, whereby studies are initially screened based on how they score against various critical criteria, which have been defined for their relevance for assessing the hazards and risks for human health. In this instance, studies that score a minimum of '1' against each of the critical criteria, regardless of how they rank according to their TAS, are prioritized as part of a Tier 1 screening and prioritization phase, which would then be followed by an expert evaluation, representing a Tier 2 level of assessment. Several key observations for strengthening future effects studies are identified, these include a need for the generation and access to standard reference materials representative of human exposure to NMPs and/or the improved characterization and verification of test particle characteristics, and the adoption of study design guidance, such as recommended by OECD.

**2.13.P-We032 Trimodal microscopy for better and faster microplastic identification IR + Raman + Fluorescence**

***Jay Anderson**, Mustafa Kansiz and Eoghan Dillon, Photothermal Spectroscopy Corp*

The problem of microplastic (MP) contamination continues to grow and is being recognized outside of scientific circles reaching the mainstream media. Articles and stories have been on "The Weather Channel", in the "New York Times", in the "LA Times", and other national and regional news outlets. California is one of the first state/localities to initiate new regulatory standards for microplastics testing aimed at measuring MP contamination in drinking and surface waters.

FTIR micro spectroscopy is the workhorse for MP testing, but suffers from spatial resolution limitations and is affected by a variety of scatter artifacts. Some studies aimed for size limits around 20µm but after rigorous evaluation had to raise those limits to 50µm.

New direct IR technologies based on IR Quantum Cascade Lasers (QCLs) are being proposed for MP testing. QCL based systems offer some advantages but they still suffer from the same fundamental problems as traditional FTIR. One study indicated that measurements with some of these new QCL systems would not be reliable below 60µm.

So a robust and easy to use MP method for testing MPs below 50µm is still lacking. But a new approach to IR micro spectroscopy called "Optical Photothermal Infrared (O-PTIR)" spectroscopy has demonstrated in several publications the ability to measure micron and even submicron MP's. O-PTIR uses a pump probe configuration and can measure mm to submicron MPs, providing FTIR transmission like spectra without the common IR scatter artifacts. O-PTIR spectra can be easily searched against commercial libraries aiding in the identification of numerous MP variants. The probe laser used in the O-PTIR technique can double as a visible source to provide simultaneous acquisition of IR and Raman spectroscopy with submicron resolution.

Coupling the well-established Nile Red fluorescent staining method, MP particles can be targeted for O-PTIR measurements. Using "Fluorescence-guided O-PTIR", the user can focus on MP's rather than other inorganics like salts, carbons etc, thus speeding up the analysis.

In this presentation we will introduce MP examples exhibiting various artifacts observed from traditional direct IR measurements, present the advantages of O-PTIR, complementarity nature of Raman, and throughput advantage using "Fluorescence-guided O-PTIR".

**2.13.V Understanding the Ecological Effects of Nanoplastics in Aquatic Environments**

**2.13.V-01 Solving Familiar Problems: Leveraging Ecotoxicity Testing Methods for Nanomaterials to Evaluate Microplastics and Nanoplastics**

***Elijah Petersen**[1] and Alan Kennedy[2], (1) National Institute of Standards and Technology (NIST), (2) U. S. Army Corps of Engineers*

The potential environmental and human health risks from microplastic (1 µm to 1 mm) and nanoplastic (<1 µm) particles (MNPs) is receiving increasing attention from scientists and the public. Most particles in the environment are likely secondary particles formed from the degradation and weathering of larger pieces of plastic. Currently, MNP hazard studies use a wide range of non-standardized methods, resulting in the low comparability of results. This hinders the generation of consistent and reliable hazard data, increases the uncertainty of risk determinations and limits the use of computational models. One approach to resolve discrepancies is using existing standardized test methods. These methods were designed for dissolved substances and to avoid physical effects from particles. However, MNPs at elevated concentrations could cause physical effects on organisms. This situation is similar to that confronted in research over the last decade studying the environmental behavior and toxicity of engineered nanomaterials (ENMs), where early publications also resulted in conflicting results. To improve the quality of the MNP data generated, a strategy may be to leverage an OECD guidance documents (GD) (317) developed for ENMs. Many of the issues in designing ENM-specific test improvements are applicable to MNPs. However, there are MNP-specific considerations that may require alterations to the methods for ENMs.

**2.13.V-02 Previous Successes and Future Opportunities for the Analysis of Environmental Nanoplastics with Pyrolysis - GC/MS**

***Meredith Evans Seeley***, *Katherine Shaw and Jennifer Lynch, National Institute of Standards and Technology (NIST)*

The chemical analysis of nanoplastics in complex matrices is a priority for human and environmental health research. Spectroscopic techniques are commonly employed to identify a plastic particle by polymer type, but require extensive sample preparation and are not applicable to particles below 1 um, limiting sample throughput and analysis of nanoplastics. Further, spectroscopic techniques require size and density approximations to estimate mass-based concentration. Mass-based plastic estimates can be useful for sub-micron particles, which may translocate into tissues but be difficult to count by particle number. Here, we review the application of pyrolysis - gas chromatography/mass spectrometry (Py-GC/MS) for mass-based estimates of polluted micro- and nanoplastics in environmental samples. Py-GC/MS is uniquely suitable for the analysis of plastics as it employs high-temperature thermal degradation of polymers to generate GC/MS amenable marker compounds (often a monomer of the polymer). Researchers have used such marker pyrolysates not just to identify polymer type, but also to quantify parent polymer concentration using external polymer standards. Uniquely, pyrolysis may also detect additive content at low (i.e., thermal desorption) temperatures, but little work has demonstrated the ability to quantify additives in plastics and the polymer simultaneously. An additional consideration for Py-GC/MS is sample preparation, as it is often necessary to concentrate plastics within a sample prior to analysis. This is particularly true for nanoplastics. The necessity for sample preparation and appropriate preparation techniques will be discussed. Following this synthesis of previous Py-GC/MS research, a path forward for establishing robust Py-GC/MS techniques will be proposed for discussion. This will include practical examples, such as the feasibility of using mass-labeled standards for an internal standard approach. Further, aspects of Py-GC/MS that have not been previously employed for the analysis of environmental plastics will be proposed (e.g., cryogenic trapping of narrowed temperature ranges). Considering the current need for nanoplastics quantification in not just environmental matrices, but also samples critical for human health (e.g., human blood), higher throughput techniques are desperately needed. As such, this presentation will open the floor to discuss the timely harmonization and improvement of Py-GC/MS approaches in the nanoplastics field.

**2.14.P Poster Only: Aquatic Toxicology, Ecology and Stress Response**

**2.14.P-We033 Suitable Dispersing Methods for Poorly Water-soluble Chemicals in Ready Biodegradability Test**

***Yoshinari Takano***[1,2]*, Kotaro Takano*[2]*, Yoshihide Matoba*[2]*, Makiko Mukumoto*[2] *and Osamu Shirai*[1]*, (1) Kyoto University, Japan, (2) Sumitomo Chemical Co., Ltd., Japan*

The ready biodegradability in accordance with the Organisation for Economic Co-operation and Development test No. 301 is required by many countries for the chemical safety management. This test shall be conducted at a high concentration even for poorly water-soluble chemicals. Hence, the bioavailability of these chemicals to microorganisms is limited under the test conditions. It appears that the biodegradability of these chemicals in the test would be suppressed in comparison with that under the realistic environment. The International Organization for Standardization 10634 (2018) mentions that the biodegradation can be accelerated by adsorbing a test chemical to silica gel with a solvent such as chloroform or by dispersing a test chemical with an emulsifier. However, chloroform residue on the silica gel or an emulsifier itself might be degraded and lead to changes of microbiota in activated sludge during the biodegradability test, which raises concerns about artificial acceleration of the biodegradation for a test chemical. Actually, biochemical oxygen demand (BOD) was increased by only adding silica gel that was treated with chloroform or Tween 85 as an emulsifier, though chloroform was well evaporated under vacuum after mixing with silica gel.

In this study, we investigated suitable solvents to mix with silica gel or suitable emulsifiers, which would not increase BOD in the test due to their biodegradation (or in the case of solvents, which would be thoroughly evaporated under vacuum), but could accelerate the biodegradation of a test chemical. As a result, we found hexane as a suitable solvent to mix with silica gel, which was well evaporated under vacuum, hardly increased BOD due to its biodegradation, but accelerated the degradation of octabenzone as a test chemical. In addition, perfluoropentanoic acid was found to be a suitable emulsifier, which was not biodegraded but accelerated the biodegradation of octabenzone. Moreover, we found methylated-cyclodextrin as a new dispersant, which was not biodegraded but accelerated the degradation of anthraquinone or octabenzone as a test chemical. These findings would be helpful to understand the biodegradability of poorly water-soluble chemicals under the realistic environment.

**2.14.P-We034 Investigation of Joint Toxicity of Pharmaceuticals and Pesticides Mixtures toward Green Microalgae Pseudokirchneriella subcapitata; Additive Model Approach**

***Josipa Papac**, Laura Keran, Marin Kovačić, Hrvoje Kušić and Ana Lončarić Božić, University of Zagreb, Croatia*

For many years now, pharmaceuticals and pesticides are frequently detected in the aquatic environment and in that context are defined as contaminants of emerging concern. Recently, a lot of concern has grown around the joint toxic effects of such compounds due to the fact that information on this emerging issue are still scarce. Such efforts are recognized by EU Commission providing an opinion on the importance of gaining such information. For the purpose of investigation and acquiring new insights within the current study three pharmaceuticals: ciprofloxacin (CIP), carbamazepine (CBZ), diclofenac (DCF) and three pesticides: alachlor (ALA), atrazine (ATZ), and simazine (SMZ) were selected in order to analyze their combined toxicity in binary mixtures. Overall, 6 single component and 18 binary mixtures toxicities were determined using green microalgae *Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*) according to standard procedure (ISO). For the description of mixture toxicities, two common mathematical models were applied: concentration addition (CA) and independent action (IA) model. Binary mixtures of pharmaceuticals indicated synergism, while mixtures that contained CBZ and CIP exhibited antagonistic behavior with the respect to the additive behavior (CA model). On the other hand, mixtures of pesticides were acting very similarly; the synergism with respect to additive behavior was observed for most combinations. The applicability of IA model as a proof of independent toxic action of the components was not confirmed in any case. Thus, CA model showed to be adequate to predict and describe the possible interactions occurring between pharmaceuticals as well as pesticides mixture, since all investigated compounds have a similar mode of action. On the term of the obtained results, we can conclude that assessing the ecological risk based only on the effects of individual pharmaceuticals or pesticides can underestimate the risk level posed by a mixture of these environmental contaminants.

**2.14.P-We035 Characterizing Metabolic Activity of Fish Liver Microsomes Towards Fluoxetine in Four Fish Species using Michaelis-Menten Enzyme Kinetics**

***Morgan Steiner**, Clemson University*

The impact of contaminants on stream fish is poorly understood. Wastewater treatment plants are not always able to remove all pharmaceuticals from their effluent, allowing these contaminants to impact streams into which they discharge. Effects of antidepressant drugs have been documented in several freshwater fish, but little is known about the metabolism of these drugs in fish. This research project used a lab experiment to measure the hepatic metabolism of fluoxetine, an antidepressant SSRI, in several fish species compared with rat liver microsomes. Four fish species were collected in a local stream in South Carolina: Bluehead Chub, Striped Jumprock, Bluegill, and Red Breast. The experiment used increasing concentrations of fluoxetine in an enzyme assay with microsomes from the different fish species. Data collection was completed using an HPLC with fluorescence detection to quantify both the amount of fluoxetine and its primary metabolite, norfluoxetine, after two hours of incubation. Enzyme kinetics were analyzed using the Michaelis-Menten model, and preliminary results of Vmax and Km values suggest an inefficiency of fish liver microsomes at metabolizing fluoxetine compared to rat microsomes, and showed additional differences between species of fish. This research would imply that fish species are more sensitive to fluoxetine than mammals and contributes to the understanding of how stream fish are impacted by pharmaceuticals in wastewater.

**2.14.P-We036 Conducting chronic sediment toxicity studies to reduce uncertainty for plant protection products**

***Alan Samel***[1]*, Nanditha Billa*[2]*, Inma Buendia*[1]*, Alex Blakey*[3] *and David Funk*[4]*, (1) FMC Corporation, (2) Eurofins Agroscience Services, (3) Syngenta, United Kingdom, (4) Stroud Water Research Center*

In Europe, it is the responsibility of the applicant to demonstrate low risk to non-target organisms to register a plant protection product. For the aquatic risk assessment, a tiered approach is used to demonstrate low risk to organism(s) that may be exposed to a plant protection product in the water *via* off-field movement (i.e., spray drift, runoff, drainage). As per guidance from the European Food Safety Authority (EFSA) Aquatic Guidance Document (2013), tier 1 single species are tested, and endpoints are adjusted using assessment factors (AF) to derive regulatory acceptable concentrations (RAC). The RACs are then compared to Predicted Environmental Concentrations (PEC) in the water or sediment to derive PEC/RAC ratios. If low risk is not demonstrated using the results from one or two surrogate test organisms, additional testing can be conducted. This can be in the form of additional single species tests in the laboratory to derive a geometric mean (Tier 2A) or a species sensitivity distribution (Tier 2B), or mesocosm studies (Tier 3). The endpoint used in the final risk assessment utilizes the results from multiple single species tests as either the HCx (hazard concentration) from a species sensitivity distribution (SSD) or the geometric mean of the study endpoints or a NOAEC from the mesocosm tests. A lower assessment factor may be applied because the number of diverse species tested reduces the uncertainty around interspecies sensitivity.

A higher tier risk assessment provided in this presentation uses endpoints from chronic laboratory studies conducted on a wide variety of freshwater and marine aquatic benthic invertebrates exposed to sediment spiked with a plant protection product. The objective is to lower the uncertainty by increasing the number and diversity of sediment organisms tested. This approach to resolving long-term risk to sediment organisms is especially challenging considering the vastly different feeding traits, life history patterns and life cycles for each organism tested. For this reason, there have been few if any attempts made to conduct a higher tier long-term multi-single species test program for plant protection product registration. In this presentation, the methodology and results of the higher tier tests are presented. The long-term study program with eight sediment invertebrate organisms was successfully conducted resulting in a higher RAC, with a greater degree of certainty.

**2.14.P-We037 Multi- and Trans-Generational Effects on Daphnia Magna of Chlorpyrifos Exposures**

***Stephanie A. Maggio***[1,2] *and Jeffrey J. Jenkins*[2]*, (1) GHD, (2), Oregon State University*

Chlorpyrifos (CPF), a broad-spectrum neurotoxic organophosphate (OP) insecticide, is subject to atmospheric and hydrologic transport from application sites to aquatic ecosystems. Across the landscape, CPF concentrations in surface water can vary spatially and temporally according to seasonal use practices.

Standardized bioassays can provide a screening-level understanding of aquatic receptor acute and chronic toxicity. However, these bioassays do not address ecologically relevant exposure patterns that may impact fitness and survival within and across generations. The aim of the present study was to evaluate the utility of a second-tier, screening-level methodology employing *Daphnia magna* multi- and transgenerational bioassays spanning four generations to investigate the effect of variable chronic CPF exposure. The multigenerational assay consisted of continuous CPF exposure across four consecutive 21-day bioassays using progeny from the previous assay for each successive generation. In the transgenerational assay, only the parent (F0) generation was exposed. For both assays, survival and reproduction were assessed across treatments and generations. Results indicated that (1) following continuous CPF exposure at ecologically relevant concentrations to four generations of *D. magna*, the highest treatment showed an apparent tolerance response for both survival and reproductive success in the F3 generation, and (2) CPF exposure to the F0 generation did not result in treatment effects in the unexposed F1, F2, and F3 generations in the apical endpoints of survival and reproduction. Employing a suite of acute and chronic bioassays, including chronic exposures spanning multiple generations, allows for a more robust screening-level evaluation of the potential impact of CPF on aquatic receptors for variable periods of exposure.

**2.14.P-We038 Monitoring of Sediment Chemical Contaminants Alongside Oyster Tissue Physiology and Gene Regulation Within the Coastal Carolinas**

***Allisan Anne Aquilina-Beck***[1]*, James Daugomah*[2]*, Mary Rider*[1]*, Dennis Apeti*[2]*, Emily C Pisarski*[1]*, Brian Shaddrix*[1]*, LouAnn Reed*[2]*, Peter B. Key*[2]*, Ed Wirth*[2] *and Marie E. DeLorenzo*[2]*, (1) CSS Inc./NOAA, (2)National Oceanic and Atmospheric Administration (NOAA)*

Natural and man-made environmental impacts can introduce chemical and biological contaminants into sensitive habitats; threatening environmental and human health. Targeted environmental monitoring of sentinel species, such as oysters, provides essential health assessments of local and regional ecosystems that could influence environmental management decisions.

NCCOS collaborated with the National Park Service (NPS) to conduct an initial environmental quality assessment within Cape Lookout National Seashore (CALO), NC. Concurrently, NOAA's longest running environmental monitoring program, Mussel Watch, collected data from established sites in South Carolina (SC) and North Carolina (NC). Sampling was conducted in July 2020 and October 2021 and consisted of: water quality parameters, nutrients, chlorophyll, turbidity, chemical contaminant analysis of sediment, and whole oyster tissue. Eastern oysters (*Crassostrea virginica*) were collected from eighteen SC and NC sites to provide an assessment of bivalve condition. Two cellular biomarkers, Lipid Peroxidation (LPx) and Glutathione (GSH), were used to assess oyster physiology. Molecular analyses of gene specific primers targeted for detecting oxidative stress, stress response to contamination, and cellular death were also used to examine fluctuations in environmental conditions from two different tissue types (gill and hepatopancreas).

Physiological data reveals tissue specific responses. Hepatopancreas data shows oysters responding to environmental stressors, however, these responses were managed through detoxification. Gill tissue data had significantly lower levels of biomarker concentrations compared to hepatopancreas. Molecular biomarkers targeting these cellular stress pathways provide stronger understanding of how combined environmental factors influence whole organism health. Preliminary sediment chemistry data indicate that pharmaceuticals, pesticides and metals were below detection limits, though some sites had variable metal levels. Contaminant levels in the oyster tissues will be used to assess relationships between chemical exposure and oyster biomarker responses.

Joining physiological and molecular techniques together with sediment and water chemistry throughout the Carolina Coast, along with controlled laboratory experiments, allows a holistic approach to understanding how exposures to changing environmental conditions influence tissue responses within this important estuarine species.

**2.14.P-We039 Environmental Persistence and Toxicity of Weathered Wildland Fire-Retardants in Aquatic Mesocosms**

***Holly J Puglis**, Christina Mackey and Michael Iacchetta, U.S. Geological Survey*

Long-term fire retardants are applied to the landscape to aid firefighters in slowing or stopping wildfires. These products make fuel sources non-flammable and can be persistent on the landscape in drought conditions. These fire chemicals are capable of weathering on riparian habitats before precipitation events flush them into streams and other water bodies. To understand the toxicity of retardants after application on the landscape, 30-60 day post hatch rainbow trout (*Oncorhynchus mykiss*) were exposed to a fire retardant, Phos-Chek® MVP-Fx or Phos-Chek® LC-95A-R, after weathering for 7-56 days on different substrates, duff, soil (high or low organic content), or gravel, under static conditions for 96 hours. Trout mortality was affected by chemical, with LC-95A-R more toxic than MVP-Fx and controls, weathering period, with toxicity generally decreasing as length of weathering period increased, and substrate type with lower mortality observed when chemicals were applied to soil compared to duff and gravel. These patterns were more pronounced in LC-95A-R treatments compared to MVP-Fx treatments. Ammonia concentrations, the primary toxic component of current use retardants, were higher in duff and gravel treatments than in the soil treatments, and generally decreased in soil treatments over time. These results suggest that wildland fire-retardants may persist in the environment and underlying substrate may alter the toxicity of these products when they enter aquatic systems.

**2.14.P-We040 The Effect of Wildland Fire-fighting Chemicals on the Reproductive Success of an Invertebrate**

***Michael Iacchetta**, Christina Mackey and Holly J Puglis, U.S. Geological Survey*

With wildfires increasing in frequency and intensity in the western United States, it is essential to understand how the chemicals used to suppress the spread of wildland fires interact with the landscape. The effect on environmental health must be understood to use these chemicals safely and effectively in emergencies. Studies have recently been published on the lethality of fire-fighting chemicals currently used by the U.S. Forest Service to freshwater organisms; however, not much has been done to investigate how these chemicals may impact the reproductive success of organisms affected by chemical intrusions. We investigated the reproductive success of *Ceriodaphnia dubia* females that were exposed to a concentration series of two current-use fire-fighting chemicals (Phos-Chek® 259-Fx and Phos-Chek® LC-95-A-R) to determine if exposure duration and concentration of the chemical altered the number of surviving neonates produced by the female. Increased concentrations of Phos-Chek® 259-Fx decreased the average number of neonates produced by the female exposed to chemical. However, exposure duration (15- or 60-minute exposure) did not affect the average count of neonates produced by the female for either chemical. Measures of reproduction are a hallmark indicator of the ecological impact of chemical products on biota because impaired reproduction has significant consequences for the structure of natural populations, as well as for the stability of the food web. Further investigation of aquatic biota found in streams native to areas with high occurrence of wildfire should be studied to determine the potential population level changes that may occur from fire-chemical intrusions.

**2.14.P-We041 Applying EcoToxChips to Identify Potential Gene Expression Markers of Exposure to Glucocorticoid Receptor Agonists**

***Jacob Collins**[1], Jenna E. Cavallin[1], Alexander R Cole[2], Kathleen Jensen[1], Michael Kahl[1], Kelvin Santana Rodriguez[1], Dan Villenueve[1] and Ashley Kittelson[1], (1) U.S. Environmental Protection Agency, (2) Baylor University*

Glucocorticoid-mediated bioactivity has been detected in North American surface waters. However, the bioavailability and in vivo potency of glucocorticoid receptor (GR) agonists in environmental mixtures remains undefined. To better understand the sensitivity of in vivo, hepatic, gene expression responses to GR agonist exposures, adult male fathead minnows were exposed for 96 h to one of three concentrations of dexamethasone (40 ng/L; 0.40 mg/L; 4.0 mg/L) or to beclomethasone dipropionate (BDP; 130 µg/L). Among several genes with GR-dependent expression in mammals, expression of only one, sgk1, was impacted at a field-relevant concentration of dexamethasone equivalents (i.e., 40 ng/L). The 384 well fathead minnow EcoToxChip v. 1.0 was then employed to identify other potential transcriptomic markers of GR-agonist exposure. Among 375 genes evaluated, six genes were differentially expressed across more than one treatment and identified as biomarker candidates for GR-agonism. The up-regulated genes were acsl5, scd, odc1, and gadd45ga, while the down-regulated genes were slc27a6 and dgat2. The aforementioned genes were identified as they were all found to be sensitive at an environmentally relevant concentration of dexamethasone (40ng/L) and responded in the same direction across all treatments of dexamethasone and BDP. Furthermore, the magnitude of induction and/or down-regulation following dexamethasone was dose-dependent. Results of this study provide additional candidate genes to consider as potential markers of in vivo exposure to GR-agonists in field studies employing caged fish. *The contents of this abstract neither constitute, nor necessarily reflect US EPA policy.*

**2.14.P-We042 Toxicity of 10 PFAS Compounds to Five Standard Marine Species**

***Nicholas T. Hayman[1]****, Molly Colvin[1], Gunther Rosen[1], Jason M. Conder[2], Wendy Hovel[2] and Jennifer Arblaster[2], (1) Naval Information Warfare Center (NIWC) Pacific, (2) Geosyntec Consultants, Inc.*

Per- and poly-fluoroalkyl substances (PFAS) are emerging contaminants of concern that are coming under increasing scrutiny at Department of Defense (DoD) sites primarily due to their use in aqueous film-forming firefighting foams (AFFF). Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) have been shown to be chronically toxic to aquatic organisms at concentrations that have been observed in some aquatic systems near PFAS sources, resulting in concerns over the environmental risk posed by these compounds. Currently, there is a need to fill in data gaps associated with paucity of effects data for marine aquatic life, limiting the assessment of ecological risks and compliance with water quality policies. In the present study, the toxicity of 10 priority PFAS compounds (PFBA, PFHxA, PFOA, PFDA, PFBS, PFHxS, PFOS, PFDS, 6:2 FTS, and 8:2 FTS) to five standard marine laboratory toxicity testing species, encompassing nine endpoints are being evaluated. These 10 PFAS compounds have been identified as important due to their presence at AFFF impacted sites. Species being evaluated include: 1) 7-d chronic survival and growth for topsmelt fish (*Atherinops affinis*); 2) 7-d chronic survival and growth for the mysid shrimp (*Americamysis bahia*); 3) 48-h embryo-larval normal development and normal survival for the Mediterranean mussel (*Mytilus galloprovincialis*); 4) 96-h embryo-larval normal development for the purple sea urchin (*Strongylocentrotus purpuratus*), and 5) 48-h germination and growth for giant kelp (*Macrocystis pyrifera*). All species were tested using standard USEPA methods. Results from the initial range finding toxicity testing will be presented and discussed and compared to the relatively few toxicity effect values available for marine species.

**2.14.P-We043 Carbon and Nitrogen Isotope Changes in Various Life Stages of the Laboratory Mayfly (Neocloeon triangulifer)**

***Jessica L. Landaverde*** *and Ryan R Otter, Middle Tennessee State University*

Aquatic emergent insects serve as an important prey items in both aquatic and terrestrial food webs. The mayfly species *Neocloeon triangulifer* has been recently developed as a model invertebrate test organism, however, much is still unknown about the differences that exist across their various life stages. In this study, we aimed to understand if changes in carbon and nitrogen stable isotopes occurred during mayfly metamorphosis and during egg deposition. We measured carbon and nitrogen stable isotopes in laboratory-reared diatoms and mayflies (*Neocloeon triangulifer*) in larvae, subimago and imago life stages collected from five different laboratories with existing cultures in the US and Canada. Due to inter-lab differences in diatoms, isotopic values in mayflies were compared to their laboratory-specific diatom values. No major changes in d $^{13}$C and d $^{14}$N were observed between life stages, however, the %N increased between larval and adult life stages. Oviposition of imagos was a significant factor, with decreases in % carbon and increases in % nitrogen being observed in pre-oviposition vs post-oviposition mayflies.

This work adds to the growing literature base on laboratory mayflies and their energetic dynamics. When considering aquatic emergent insects for use in food web studies, the different life stages may not have an impact on stable isotopes, however, life stage and oviposition status in adults will need to be taken into consideration.

**2.14.P-We044 Seasonal Variation in Size, Stable Isotopes (Carbon & Nitrogen), and Fatty Acids in Tetragnathid Spiders**

***Jessica L. Landaverde*** *and Ryan R Otter, Middle Tennessee State University*

Riparian spiders are used in ecotoxicology as bioindicators of aquatic to terrestrial transfer of energy and bioaccumulative contaminants through the insect mediated contaminant pathway. Spiders in the family *Tetragnthidae* are particularly of interest because of the high amount of emergent aquatic insects in their diet. These spiders have become more well studied in recent years; however, the changes in their diet and size throughout a year have not been investigated. In this study, our objective was to determine if variation in size, carbon and nitrogen stable isotopes, and the fatty acid biomarker eicosapentaenoic acid (EPA) occurs in tetragnathid spiders throughout a year. Spiders were sampled within a 100m reach of the East Fork Stones River in Cannon County, TN twice a month between April and November 2021. It was found that spider total mass and body measurements steadily increased from April to September, then decreased in October. It was also found that carbon and nitrogen stable isotopes were consistent over time and that the %EPA of total fatty acids in spiders varied greatly, with spikes in May and August. This work adds to the growing literature on the use of tetragnathid spiders as bioindicators. The implications of this research show that seasonality may not be a significant factor when considering stable isotope or EPA as food web tracers.

**2.14.P-We045 Assessing the Efficacy of Contaminant of Emerging Concern Removal by Membrane Bioreactor in Wastewater Treatment Utilizing Fathead Minnow (Pimephales Promelas) Exposure**

***Charles Christen****, Alissa VanDenBoom, Molly Lovsness and Heiko Schoenfuss, St. Cloud State University*

Wastewater comes from a variety of sources from industrial, commercial, and residential areas. Each source adds contaminants of emerging concern (CECs) that can interact with an organism's cellular pathways and metabolic processes. Wastewater treatment plants (WWTP) are built to remove macro pollutants and bacterial nutrients through two-stage processes but are not optimized for CEC removal. Secondary treatment technologies range from well-established oxidative treatments to more recently developed membrane bioreactors (MBR). Oxidative treatments use agitators to promote bacterial growth and nutrient removal, while MBRs uses a similar biological treatment but add membrane filtration. The efficacy of MBR in removing CECs from wastewater is unknown. The objective of this study was to compare the CEC removal efficacy of MBR to that of oxidative treatment through 21-day exposure of fathead minnows (*Pimephales promelas*) assessing endpoints related to contaminant exposure. Fathead minnows were exposed via a flowthrough system to treatments including a negative control, oxidative treatment, and MBR effluent. Following exposure, plasma, liver, gonad, gills, and digestive tract were collected. Tissues were analyzed for indicators of CEC stressors in cellular pathways and metabolic processes. Analytical chemistry confirmed CEC removal rates across treatment steps. The entire experiment was repeated once.

**2.14.P-We046 Experimental Arena Size Influences Larval Zebrafish Photolocomotor Behaviors and Response Thresholds Following Exposure to the Neurostimulant Caffeine**

***Lea Marie Lovin****, Kendall Rose Scarlett, Abigail Henke, Jaylen Lesean Sims and Bryan W. Brooks, Baylor University*

Behavioral experiments with fish and other species are useful and sensitive tools for studying a wide variety of contaminants. However, many behavioral endpoints can be highly variable, so understanding how experimental parameters influence detection of behavioral changes, including responses to environmental stressors, represents a key issue in comparability and applications of these experimental designs within and among species. Previously multiple sizes of experimental arenas have been used to measure photolocomotor changes with age/size and for diverse endpoints under investigation. However, there remains a need to understand whether behavioral responses are influenced by experimental arena size and if they differ after exposure to a contaminant. Thus, we initially defined baseline swimming profiles of larval zebrafish under light and dark conditions using a ViewPoint Zebrabox across different plate sizes (96, 48, 24, 12, and 6 well). We then performed studies with the common neurostimulant caffeine (0.003 – 112 mg/L) and zebrafish, and analytically verified caffeine treatment levels. Following caffeine exposure, we observed behavioral responses (i.e. distance travelled, number of discrete movements, and duration of movements at different speed thresholds) using these different observation areas, using these five plate types with different well sizes. We found that swimming total distance of naïve fish increased logarithmically with increasing size of the observation area while standard deviation initially increased and then decreased with increasing plate well size. Under light conditions, significant behavioral responses to caffeine were not observed in the smallest wells, but were then more pronounced with larger arena size. Similarly, we observed significant stimulatory responses, including bursting duration, count number and distance traveled, only in the largest arena size, but these effects were not identified in smaller wells. Our results indicate that increasing well size leads to higher movement levels, and performing larval zebrafish larval behavioral assays in smaller observation arenas may not detect or underestimate photolocomotor response thresholds to chemical contaminants. Understanding these changes can improve comparability among experimental designs with different arena sizes and demonstrates the importance of characterizing potentially confounding variables during behavioral assays.

**2.14.P-We047 Effect of N-Butyl Benzenesulfonamide (NBBS) and Triphenyl Phosphate (TPhP) on the Sperm Count of Echinogammarus marinus**

***Bidemi Green-Ojo****, Matthew Parker, Lena Grinsted and Alex Ford, University of Portsmouth, United Kingdom*

Chemical exposure can be linked to adverse reproductive effects, including reducing sperm count and/or sperm quality, which are significant criteria for determining individual to population-level effects. N-Butyl benzenesulfonamide (NBBS) and Triphenyl phosphate (TPhP) are plasticizers commonly used in commercial and consumer products that may potentially be harmful to humans and animals in different capacities. Due to the recent use of these compounds as replacement plasticizers, their impact on sperm parameters, mainly in invertebrates, is widely unknown. This study investigated the impact of subchronic exposure of NBBS and TPhP on the sperm count of a coastal marine amphipod, *Echinogammarus marinus*. Animals were exposed to low and high concentrations of both compounds for 14 days. At the end of the exposure, animals were anaesthetized and weighted while the sperms were collected and counted under a fluorescence microscope. This poster will present (i) the relationship between the weight of the amphipod and the sperm count and (ii) report the sperm count and establish a dose-response relationship. The findings of this study will highlight the use of sperm parameters as a valid reproductive endpoint in environmental risk and monitoring practices.

**2.14.P-We048 Evaluation of Toxicity of Contaminated Sediment in the Kanawha River, West Virginia, to Unionid Mussel (Fatmucket, Lampsilis siliquoidea) and Common Test Benthic Organism (Amphipod, Hyalella azteca)**

***Chris D. Ivey***[1]*, Ning Wang*[1]*, James Kunz*[1]*, Jeff A. Steevens*[1] *and Kathleen Patnode*[2]*, (1) U.S. Geological Survey, (2) U.S. Fish and Wildlife Service*

Industrial discharges and unregulated releases containing polyaromatic hydrocarbons (PAHs), volatile organic compounds (VOCs), and metals have entered the Kanawha River surrounding Blaine Island, South Charleston, West Virginia (WV). The Kanawha River supports 8 federally endangered mussel species and 2 proposed mussel species. The potential toxicity of contaminant mixtures in Kanawha River sediment to native mussels is unknown. Sediment samples collected from an upstream reference site (001), a test site (002) at the proposed pipe relocation area, and a test site at the known area of contamination (003) were used to assess the effects of contaminated sediment on the survival and growth of a unionid mussel (Fatmucket, *Lampsilis siliquoidea*) and a commonly tested benthic invertebrate (amphipod, *Hyalella azteca*) using ASTM standard 4-week sediment toxicity test methods. A sediment control and a sand control were also included in the sediment toxicity testing. In addition, a longer term (12-week) sediment test with the mussel was conducted to evaluate potential effects in a longer test duration. Finally, a 4-week sediment toxicity test with juvenile fatmucket was performed in a serial dilution of the contaminated sediment 003. Results showed that measured concentrations of contaminants (PAHs, VOCs, metals) in sediment 003 were consistently greater than those in sediments 001 and 002. Mean survival of mussels and amphipods in all controls and the reference site ranged from 93 to 100% whereas mean survival of both test species in the sediment 003 was 0% in the 4- and 12-week sediment toxicity tests, indicating that the contaminated sediment was extremely toxic to the amphipods and mussels. However, mean survival and growth of both species in the sediment 002 were not significantly different from the reference sediment 001.The sediment 003 dilution exposure indicated that mean survival and biomass of mussels in ≥6.25% sediment were significantly reduced relative to the control sediment, with a 25% inhibition concentration of 4.1% sediment 003 for survival and 3.6% for biomass. These results will help inform decisionmakers on how to best protect freshwater mussels occurring in the Kanawha River.

**2.14.P-We049 Comparison of Two Toxicogenomic Analysis Tools, RNAseq and EcoToxChip, Using Fathead Minnows (Pimephales promelas) Exposed to Paroxetine**

***Michael Edward Ellman***[1]*, Kendra Bush*[2]*, Kevin Flynn*[2]*, Monique Hazemi*[2]*, John Hoang*[2]*, Michelle Le*[2]*, Kelvin Santana Rodriguez*[2] *and Daniel L. Villeneuve*[2]*, (1) Oak Ridge Institute for Science & Education at US EPA, (2) U.S. Environmental Protection Agency*

Short-term testing employing transcriptomic endpoints has been proposed as an alternative to long-term exposure and direct observation of apical effects for generating lower bound estimates of *in vivo* effect concentrations and mechanistic insights to inform subsequent tiers of testing. While RNA sequencing (RNAseq) is now the dominant technology for evaluating whole transcriptome response, the size and complexity of the data sets can make analysis and interpretation challenging for non-experts. Here we examine whether analysis of a targeted gene set using real time polymerase chain reaction array technology (EcoToxChip) could serve as a more accessible, but nonetheless informative, alternative to RNASeq. RNA was extracted and analyzed from whole body larval fathead minnows (*Pimephales promelas*) exposed to one of 11 concentrations of paroxetine or a control treatment (n=8 per treatment) for 24 h in a 96 well plate. To enable comparison of the RNASeq and EcoToxChip platforms, concentration-response analyses were also performed. RNASeq allowed for quantification of 20,145 transcripts, while the EcoToxChip focused on 375 genes selected for their toxicological relevance. Based on the full experimental design, 77-2,292 differentially expressed genes were detected per treatment group by RNASeq. However, using a smaller sample set selected for the platform comparison (i.e., n=3 or 4 individuals per treatment; 6 treatments), a maximum of 8 transcripts (0.04% of the measured transcriptome) were detected as differentially expressed via RNAseq. In contrast, for EcoToxChip results analyzed using the online EcoToxXplorer software, 17 transcripts (4.5% of the selected genes) were identified as differentially expressed. Among the 20,145 transcripts measured by RNAseq, concentration response curves were successfully fit for 335 genes (1.6%); the median and 10th centile of the gene specific benchmark doses (BMD) were 0.8 and 0.5 mg/L, respectively. Among the 375 genes evaluated via EcoToxChip, regression models could be fit for 5 genes (1.3%); a 10th centile was not determined, but the median BMD was 0.2 mg/L. These results provide insight into the strengths and limitations of the EcoToxChip platform versus RNAseq, as well as important experimental design considerations. *The contents of this abstract neither constitute, nor necessarily reflect, US EPA policy.*

**2.14.P-We050 Fitness Effects of Metal Accumulation in Invasive Japanese Mysterysnails**

***Brooke Anne Johnston***[1]*, Lynne Beaty*[2]*, Adam Martin Simpson*[2] *and Sam Nutile*[2]*, (1) Penn State University - Erie, (2) Penn State Behrend*

Originally from Asia, Japanese mysterysnails (JPM) (*Cipangopaludina japonica*) have spread to eastern and midwestern waters of the United States. Due to their large size and filter feeding habits, JPM accumulate metals from their environment into their shells and tissues. As with other invasive species, the toxic effects of metal accumulation on JPM fitness are unknown. Therefore, the objective of this study was to examine metal accumulation within the tissues and shells of JPM and determine the effects of this accumulation on individuals' fitness. Snails, water, and sediment were collected from two locations in Pennsylvania, Pymatuning Reservoir and Lake Arthur, known to have populations of JPM. Snails collected from each location were returned to laboratory facilities and behavior was monitored by documenting the activity (i.e., active/not active) of the snails every five minutes for two hours. After a 24 h depuration period, snails were frozen and dissected to document shell and tissue weight, measure shell thickness, and isolate tissues for determination of metal bioaccumulation. Metal concentrations within the snails, water, and sediments were then determined using acid digestions and quantification via an inductively coupled plasma mass spectrometer and correlated to metrics of snail fitness. Of the metals detected in the snails and environmental matrices (i.e., nickel, selenium, manganese, copper, barium), only barium had a significant effect on the fitness of JPM, causing reduced activity in individuals with higher tissue concentrations. Little is known about the effects of environmental contaminants on invasive species, but this information is essential to effective management of invasive species in relation to population density and spread of invasive species to new habitats. As snails are known to be particularly sensitive to metal contaminants, this study aims to fill a knowledge gap related to invasive species management and ecotoxicology.

**2.14.P-We051 Identifying Native Pennsylvania Aquatic Plants for use in Bioremediation of Metals**
***Noah Janes****, Sam Nutile, Adam Martin Simpson and Lynne Beaty, Penn State Behrend* |
Phytoremediation of metal contaminants is apt approach to reducing bioavailable metal concentrations in terrestrial and aquatic habitats. Many plant species, specifically those found in wetland habitats, are capable of accumulating high concentrations of metals with little toxicological effect. Phytoremediation, however, can be limited by the need to be conscious of preventing the introduction non-native plant species to new habitats as the spread of non-native species can undermine the advantages of metal removal. Identifying additional plant species that are native to a wide range of areas and capable of accumulating significant metal concentrations, however, may expand the applicability of metal phytoremediation by generating a register of metal accumulating plants. Therefore, the objective of the current study was to document metal accumulation in native plant species around Presque Isle State Park, Pennsylvania for potential use in phytoremediation. Within Presque Isle State Park, haphazardly placed 1 m^2 quadrats were used to determine metal accumulation by native plants. Plants within each quadrat were identified and tissue samples, along water and sediment samples, were collected for metal analysis. Metal concentrations in plant tissue, water, and sediment samples were determined through acid digestion and analysis via inductively coupled mass spectrometry. While plant tissue sample processing is ongoing, the diversity of plants found within Presque Isle State Park, including Coontail (*Ceratophyllum demersum*), Common Duckweed (*Lemna minor*), and Common Waterweed (*Elodea canadensis*), and the presence of cadmium, copper, nickel, zinc, and lead in sediment would suggest some resiliency to metal accumulation. Identifying a wide range of plants that could function in phytoremediation would help extend the applicability of this methodology in different habitats and locations within the United States by utilizing plants native to contaminated areas. Without the threat of invasive species introductions, more effective metal removal can occur, and this project represents an initial step towards this goal within Pennsylvania.

**2.14.P-We052 Maternal Transfer of Metals in Invasive Mysterysnails**
***Jessica Pengilly****, Ann-Marie Millunzi, Adam Martin Simpson, Sam Nutile and Lynne Beaty, Penn State Behrend*
Aquatic snails are known to accumulate metals into their tissues and shells based on environmental exposure. Due to the presence of a calciferous shell, snails are capable of offloading metal contaminants into their shells during development, reducing concentrations in more sensitive tissues. This process may be exacerbated in snail species, such as invasive Chinese (/Cipangopaludina chinensis/) and Japanese (/C. japonica/) mysterysnails, which are ovoviviparous. The formation of juvenile shells within the brood pouch of the mother may allow for extensive maternal transfer of metal contaminants to offspring, but little is known about this process in these invasive species. Therefore, the objective of the current study was to quantify maternal transfer of metal contaminants in Chinese and Japanese mysterysnails collected from Pennsylvania waters. Invasive snails were collected from five locations around Pennsylvania, including Presque Isle State Park, Lake Arthur, Pymatuning

Reservoir, Lake Canadohta, and Lake Pleasant. After a 24 h depuration period, adult snails were dissected and gravid females were isolated for analysis. Maternal tissues and offspring within the brood pouch were digested and metal concentrations were quantified via an inductively coupled mass spectrometer. Sample processing is currently ongoing, but preliminary results suggest significant offloading of metals from mother to offspring, suggesting significant metal transfer of metal contaminants. Offloading of metals from mother to offspring through shell development may have relevant implications for population dynamics of invasive snail populations in non-native waters through toxic effects on juvenile snails. Replacement of calcium with other metal ions in calciferous shells may reduce shell integrity, reducing survival of offspring, altering population dynamics. Similarly, female snails may reduce their overall body burden, reducing potential toxic effects associated with metal accumulation. Better understanding of maternal transfer in invasive mysterysnails is therefore required for effective management of these invasive species.

**2.14.P-We053 Feeling a S-midge Depressed?: Intergenerational Effects of Selective Serotonin Reuptake Inhibitors (SSRIs) on Chironomus dilutus**

***Lynne Beaty**, Kaylin Currier, Dachoda Jones and Sam Nutile, Penn State Behrend*

Selective serotonin reuptake inhibitors (SSRIs) have experienced increased consumer use in recent years. Like other pharmaceuticals, SSRIs often bypass wastewater treatment facilities and can be dispersed into adjacent water bodies. Although SSRIs have documented within-generation effects on aquatic organisms, whether—and how—the effects of SSRIs transcend generations are poorly understood. This study aims to investigate the intergenerational effects of two SSRIs—sertraline and fluoxetine—on the survival, growth, and reproduction of *Chironomus dilutus* midges. Using a full factorial design, sertraline and fluoxetine were applied independently and in combination to larval midges for two generations and compared to controls. Sex ratios, reproductive effort, and survival, documented as successful emergence to the adult stage, were recorded for each generation and the effects of parental (F1) and offspring (F2) treatments were compared. Exposure to SSRIs did not impact F1 midge survival, sex ratios, or reproduction. In contrast, F1 exposure to SSRIs significantly impacted F2 midge emergence, sex ratios, and reproduction. In particular, F2 midges whose parents had been exposed to both sertraline and fluoxetine experienced reduced emergence success, a more male-biased sex ratio, and reduced reproduction. This study will add to the growing body of literature documenting intergenerational effects of SSRI contamination and, more broadly, how pharmaceutical contamination influences aquatic ecosystems.

**2.14.P-We054 Selection Preference of Hyalella azteca in Response to Environmental Contaminants**

***Miranda Johns**, Sam Nutile, Adam Martin Simpson and Lynne Beaty, Penn State Behrend*

Non-target organisms in aquatic environments are consistently exposed to contaminants due to runoff of widely used pesticides. These organisms are not only affected by the lethal effects of these contaminants, but sublethal effects that often occur at much lower concentrations. Sublethal effects on growth, reproduction, and physiology are well documented within the scientific literature, but sublethal effects related to behavior are severely understudied, yet changes in behavior may result in population level effects. Therefore, the objective of the current study was to utilize a previously designed behavioral assay to monitor the avoidance behavior of *Hyalella azteca* when exposed to four different concentrations of bifenthrin based on acute toxicity. A series of toxicity tests were conducted to determine the 48-h lethal concentration 50 ($LC_{50}$) for *H. azteca* exposed to bifenthrin. Based on this lethal concentration, a behavioral assay was used to observe if *H. azteca* can avoid contaminated sediment at the $LC_5$, $LC_{10}$, $LC_{25}$, and $LC_{50}$. For each bifenthrin concentration, thirty *H. azteca* were placed individually into a custom behavioral arena and given a choice of clean or bifenthrin contaminated sediment. Over a ten-minute observation period, the time an individual *H. azteca* spent in clean versus contaminated sediment was recorded. Even at the lowest bifenthrin concentration, *H. azteca* demonstrated a strong preference for uncontaminated sediment. The activity of *H. azteca*, documented by the number of times individuals moved between arena zones, was significantly increased at lower bifenthrin concentrations ($LC_5$, $LC_{10}$) compared to higher concentrations ($LC_{25}$, LC*50*), suggesting a reduced ability to detect bifenthrin at lower concentrations. The results of the current study demonstrate the ability of *H. azteca* to actively avoid contaminated sediments at sublethal concentrations, which may impact environmental surveys of contaminated ecosystems as the absence of a species may not be due to a mass die-off but a migration to less contaminated areas. Avoidance of contaminated sediments could have population level effects through movement to poorer habitats or increased predation risk. This study further indicates the need to incorporate behavioral assays in toxicological assessment of legacy and emerging contaminants.

**2.14.P-We055 Fate and Effects of Metformin on a Boreal Lake Ecosystem**
***Nicholas Blandford***[1]*, Vince Palace*[2]*, Mark McMaster*[3]*, Kristin Nielsen*[4]*, Joanne L. Parrott*[3]*, Karen Kidd*[5]*, Mehran Alaee*[3]*, Thomas Clark*[3]*, Jessie Cunningham*[3]*, Abby Wynia*[3] *and Erin Ussery*[3]*, (1) University of Manitoba, Canada, (2) IISD Experimental Lakes Area (IISD-ELA), Canada, (3) Environment and Climate Change Canada, (4) The University of Texas at Austin, (5) McMaster University, Canada*
The type-2 diabetes drug metformin is among the most commonly prescribed pharmaceuticals in the world. Consequently, metformin is also among the most abundant pharmaceutical contaminants in the environment and has been detected in the ng-µg $L^{-1}$ range in various surface waters and wastewater effluents. However, little is known about the environmental fate of metformin and the risk it poses to aquatic ecosystems. An 8-week in-lake mesocosm (i.e. limnocorral) experiment was conducted at the IISD – Experimental Lakes Area (IISD-ELA) to investigate how experimentally added metformin would distribute in a boreal lake ecosystem and investigate the potential for environmentally relevant concentrations of metformin to have adverse effects upon biotic communities in affected ecosystems. Four replicate limnocorrals (2 m diameter; ~3000 L) were each assigned a treatment of 0, 4, and 40 µg $L^{-1}$ metformin. Limnocorrals captured the biotic and abiotic components of a boreal lake ecosystem. Additionally, 10 wild fathead minnows (*Pimephales promelas*) were added to each limnocorral. Microbial, phytoplankton, zooplankton, and benthic invertebrate communities were monitored throughout the exposure period. No adverse effects were observed on these communities for the 8-week exposure period. However, metformin concentrations were quite stable in the water column, with significant concentrations persisting over 1-year after the exposure period. Additionally, no significant effects were observed on adult fathead minnow fish health endpoints such as length, body weight, condition, and both liver and gonadosomatic indices. As well, no significant difference in hematocrit or gonad histology in adult fathead minnows was observed at the close of the 8-week study. These results inform on the chronic ecosystem level effects of environmentally relevant concentrations of metformin and ultimately contribute to the understanding of the fate and environmental risk of metformin.

**2.14.P-We056 The presence, distribution, and concentration of trace metals in the Potomac River near a Virginia coal ash repository**
***Elizabeth Tyler****, Rachael Harrington, Catherine Crowell, Leanna Giancarlo, Ben Odhiambo Kisila and Tyler Edward Frankel, University of Mary Washington*
The Chesapeake Bay watershed contains several coal-burning power stations located along its waterways. Coal ash, one of the largest forms of industrial waste, is primarily produced by power stations and disposed of in coal ash repositories. Known to be heavily enriched with trace metals, these contaminants are then able to enter surrounding aquatic environments. Few studies have examined trace metal contamination within the Potomac-Shenandoah watershed stemming from these repositories. Thus, the goal of this study was to assess the spatial and temporal distribution of trace metals in sediments and surface waters adjacent to the Possum Point power station (Quantico, VA). Water and sediment samples (grab and core) were collected from several sites upstream, midstream, and downstream from the station. Trace metals from each sample were extracted and analyzed using ICP-OES (inductively coupled plasma optical emission spectroscopy) for the presence and concentration of Al, As, Cd, Ca, Cr, Cu, Fe, Pb, Mg, Mn, Se, and Zn. Cores were sectioned at 2cm intervals and sediment chronology established using $^{210}Pb$. While this study is still ongoing, we expect to find elevated concentrations of these metals midstream and downstream from the powerstation. Based on chronological data, we also expect to observe enriched trace metal deposits that occur after the coal ash repositories were created.

This study will provide vital information regarding the prospective impacts of coal-burning repositories on the release and mobilization of trace metal contaminants within aquatic ecosystems in the Chesapeake Bay region.

### 2.14.P-We057 Oxidative Stress Response in Blue Mussel (Mytilus edulis) Exposed to Ciprofloxacin, Diclofenac, and Binary Mixtures

***Jason Tyler Magnuson**, Lilja S.M. Pedersen, Hans Kristian Brekken, Eli Drange Vee and Daniela Maria Pampanin, University of Stavanger, Norway*

The global increase in consumption of pharmaceuticals and residues detected in the environment raises concern to aquatic organism health. The discharge of pharmaceuticals from wastewater treatment plants is a predominant source of those compounds to the environment due to an incomplete removal prior to release in ecosystems. However, the impact of pharmaceutical mixtures to marine species, such as the blue mussel (*Mytilus edulis*), is limited, with a majority of previously conducted work assessing the effects of individual compounds. Two commonly prescribed pharmaceuticals in Norway that have known risks to marine invertebrates, ciprofloxacin and diclofenac, were selected in this study. Mussels were exposed to 0.5, 1.0, and 10 µg/L of ciprofloxacin, diclofenac, or as a binary mixture for 7 days to assess potential oxidative stress responses by determining glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), and lipid peroxidation (MDA) activity in the digestive gland. There were significant differences in GPx activity between mussels exposed to ciprofloxacin or diclofenac individually relative to binary mixtures. SOD activity was significantly lower in diclofenac exposed mussels relative to binary mixtures, with significantly greater CAT activity in mussels exposed to 0.5 µg/L of ciprofloxacin + diclofenac, relative to exposures of each individually. Interestingly, there was a greater incidence of lipid peroxidation in mussels exposed to 1.0 µg/L ciprofloxacin relative to either diclofenac or the binary mixture. This data suggests that an ecologically and economically important marine bivalve exposed to environmentally relevant concentrations of ciprofloxacin and diclofenac can exhibit an increased incidence of oxidative stress, with a greater response noted in mussels exposed to a binary mixture, a more environmentally realistic scenario than individual pharmaceutical compounds.

### 2.14.P-We058 High-Throughput Behavioral Effects of Multiple PFAS Chemicals in Larval Pimephales promelas

***John Hoang**[1], Michelle Le[1], Monique Hazemi[1], Kendra Bush[1], Michael Edward Ellman[2], Kevin Flynn[1], Brett R. Blackwell[1], Emma Stacy[1] and Daniel Villenueve[1], (1) U.S. Environmental Protection Agency, (2) Oak Ridge Institute for Science & Education at US EPA*

Due to their prevalence in the environment and concerns regarding potential toxicity, there has been a surge in public support to further research per- and polyfluoroalkyl substances (PFAS) and better understand their fate, toxicokinetic properties, and potential adverse effects. The present study used a 24-hour high throughput assay to evaluate the effects of 22 PFAS on locomotor responses in larval *Pimephales promelas.* Five-day old *Pimephales promelas* were exposed to individual PFAS at 0.03, 0.1, 0.32, 1, 3.17, 10, 31.67, or 100 µM for 24 hours in deep well, 96 well microplates. Additionally, exposures with potential positive control chemicals, caffeine and ethanol, were performed. Following exposure, plates were placed in a *DanioVision* observation chamber; behaviors, including percent activity and distance traveled, were recorded using high content video over a 5-minute light on-off protocol utilizing *EthoVision XT 15* detection software. The data were subsequently exported and processed through a custom data analysis pipeline in R to measure relevant endpoints, such as average percent activity when the light was on. While the majority of PFAS chemicals tested showed little to no effect on behavioral endpoints, potassium perfluorooctanesulfonate and 3H-Perfluoro-2,2,4,4-tetrahydroxypentane altered behavioral responses in a dose-dependent manner. Potassium perfluorooctanesulfonate significantly increased photomotor responses beginning at 10 µM, whereas 3H-Perfluoro-2,2,4,4-tetrahydroxypentane significantly decreased responses at 31.67 µM. In relation to survival, perfluorooctanesulfonate had no mortality within any of the tested concentrations, while the 100 µM 3H-Perfluoro-2,2,4,4-tetrahydroxypentane concentration elicited 13% mortality across 3 replicate plates. Caffeine showed promising results as a possible positive control, whereas ethanol will require further testing. Overall, this assay demonstrated that while most PFAS studied had little effect on larval *Pimephales promelas* behavior, certain chemicals did produce a response that could not be captured by the mortality endpoint alone. Results of these behavioral assays will be further compared with both mortality and transcriptomics-based points of departure to provide novel information on the potency of the tested PFAS and potential insights into structural features that may lead to toxicity in fish. *The contents of this abstract neither constitute, nor necessarily reflect, official US EPA policy.*

**2.14.P-We059 Critical Review and Recommendations to Improve the Quality and Reproducibility of the U.S. EPA Chronic Mysid Shrimp Testing Guideline: Update on a CropLife America and CropLife Europe Project**

*Alan Samel[1], Jane Staveley[2], **Margaret Fleming**[2], Alan J. Jones[3], Eric Bruns[4], Adric Olson[5], Cliff Habig[6], Bridget O'Neill[7], Audrey Bone[4], Kevin Henry[8], Gwendolin Kraetzig[3], Theodore Valenti[9], Maike Habekost[5], Michelle Blickley[7], Eric Peterson[9], Joseph Marini[01], Lee Sayers[01], Amanda Milligan[11], Sean Gallagher[11] and Suzanne Schneider[11], (1) FMC Corporation, (2) Exponent, (3) ADAMA Ltd., (4) Bayer AG - Crop Science Division, Germany, (5) BASF Corporation, (6) Compliance Services International (CSI), (7) Corteva Agriscience, (8) Tessenderlo Kerley, Inc., (9) Syngenta, (10) Smithers, (11) Eurofins Agroscience Services*

The U.S. Environmental Protection Agency Office of Prevention, Pesticides, and Toxic Substances (OPPTS) 850.1350 Mysid Chronic Toxicity Test (draft, 1996) is conditionally required for the registration of pesticides in the U.S. Although this study is not required by regulatory agencies outside of North America, results may have an impact on product registrations internationally. Valid completion of this study can be challenging as the test guideline (TG) provides limited technical information on study design and conduct. Achievement of test organism performance criteria as currently specified is difficult, which often results in the need to repeat studies.

The objectives of a project undertaken by CropLife America and CropLife Europe are to identify: 1) areas of inconsistency between the draft OPPTS and other available TGs; 2) areas in the draft OPPTS TG that require additional clarification; and 3) areas in the draft OPPTS TG that are not practical or have uncertain scientific relevance with respect to the objectives of the study design.

Over one hundred mysid shrimp chronic toxicity study reports finalized between 1987 and 2021 were assembled from 18 sponsors representing 6 testing laboratories. A database was created for negative and solvent control endpoints including adult survival, day of first brood release, number of reproductive days, number of young, length and dry weight of adults (by sex), duration of second generation (G2) exposure, G2 survival, and any other G2 observations. Adult exposures typically lasted 28 days while G2 exposures, included in almost 2/3 of the studies, typically lasted 4 days. Average adult and G2 survival exceeded 85%. The number of young produced averaged more than 1 per reproductive day. The number of young produced per surviving female averaged 16. However, variability was high for reproductive endpoints. More than 90% of studies included adult length measured at test termination and adult dry weight was reported in approximately 75% of studies. No-observed-effect-concentrations (NOECs) were most commonly based on reproductive endpoints, followed by adult growth endpoints and adult survival. NOECs were rarely based solely on a G2 endpoint.

This presentation will provide an overview of the aggregated data from the toxicity study reports, a comparison of observations with current TG criteria, and recommendations for clarifying sections of the TG and relevant acceptability criteria.

**2.14.P-We060 Assessing the Presence and Concentration of Trace Metals in Surface Waters, Aquatic Plants, and Fish Adjacent to a Virginia Coal Ash Repository**

***Carolyn Willmore**, Leanna Giancarlo, Ben Odhiambo Kisila, Rachael Harrington and Tyler Edward Frankel, University of Mary Washington*

Coal ash (CA) is an industrial waste that has been shown to contain several neurotoxic trace metals including cadmium, arsenic, mercury, and lead. These contaminants are then able to leach into surrounding waterways and cause undesirable ecosystem effects. According to the Virginia Department of Environment, there are currently twenty-eight CA repositories that are situated near waterways which act as tributaries for the Chesapeake Bay, many of which are located adjacent to coal-fired powerplants. While previous studies have examined the presence and concentrations of these trace metals in surface water and sediments surrounding these industrial sites, our understanding of whether they bioaccumulate in aquatic flora and fauna inhabiting these waters remains relatively poor. As such, this study examined the presence and concentration of twelve different trace metals (Al, As, Cd, Ca, Cr, Cu, Fe, Pb, Mg, Mn, Se, Zn) in plant and fish tissues collected near the Possum Point Powerplant (Dumfries, VA). Male and female Banded killifish (*Fundulus diaphanus*) were collected from each site and epaxial muscle, gonads, and brain tissue collected via gross dissection. To assess differences in reproductive status, whole body and gonad weights were obtained to calculate the gonadosomatic index of each individual. Whole hydrilla spp. were collected from each site. All collected samples were then oven-dried and trace metals extracted using a 65% nitric acid : 30% hydrogen peroxide solution. Once extracted, concentrations of each element were assessed using ICP-OES. While this study is still ongoing, we expect to find elevated concentrations of several neurotoxic trace metals in fish and plants collected adjacent to the location compared to associated water and sediment samples, with increased bioconcentration exhibited by Hydrilla spp. The results of this study will provide a more detailed understanding of the large-scale impacts of this CA repository on adjacent waterways via bioaccumulation and serves as support for future studies examining impacts on higher tropic levels.

**2.14.P-We061 Selenium:Mercury Molar Ratios in Tissues from Young-of-the-Year and Juvenile Sharks in Texas Bays**

***Natalie Pitman**[1], Weston Nowlin[1], Faye Grubbs[2], Brian Jackson[3] and Jessica Dutton[1], (1) Texas State University, (2) Texas Parks and Wildlife Department, (3) Dartmouth College*

Mercury (Hg) can accumulate to high concentrations in sharks, potentially resulting in adverse health effects. Selenium (Se) has an antagonistic relationship with Hg and may have a protective effect against Hg toxicity if the Se:Hg molar ratio is >1:1. Muscle and liver Se:Hg molar ratios have been investigated in adult sharks, however, little is known about Se:Hg molar ratios in tissues of young-of-the-year (YOY) and juvenile sharks due, in part, to the difficulty of acquiring samples. This study investigated the Hg and Se concentrations, and Se:Hg molar ratios in dorsal muscle, heart, brain, kidney, and liver of YOY and juvenile bull sharks (*Carcharhinus leucas*), blacktip sharks (*Carcharhinus limbatus*), and bonnethead sharks (*Sphyrna tiburo*), in four bays along the Texas coast (Sabine Lake, Aransas Bay, Corpus Christi Bay, and Lower Laguna Madre). Sharks were collected during the Texas Parks and Wildlife Department 2020-2022 fall and spring gill net seasons (n = 30 per species per bay), tissues were dissected and freeze-dried, and the Hg concentration measured using a direct mercury analyzer (DMA-80) and the Se concentration measured using microwave acid digestion and Inductively Coupled Plasma Mass Spectrometry (ICP-MS). There was intra- and interspecies variability in Hg and Se concentrations and Se:Hg molar ratios. Overall, Hg concentrations were greatest in dorsal muscle and heart, and Se concentrations were greatest in kidney and heart. The Se:Hg molar ratios were >1:1 in all tissues, indicating that Se may have a protective effect against Hg toxicity in immature sharks. Se:Hg molar ratios in each tissue decreased with increase in Hg concentration, however, no clear relationship between Se:Hg molar ratios and body length was observed, with some tissues and species showing an inverse relationship whereas others showed no relationship. High tissue Se:Hg molar ratios during early life stages (e.g., heart >100:1 and brain >150:1) could reduce the adverse effects of Hg on growth and development, however, the Se:Hg molar ratio at which Se is protective against Hg toxicity (e.g., 1:1, 5:1, or greater) is not known, highlighting the need for further research in taxa that have higher Hg concentrations due to their reproductive strategy, long-life span, and high trophic position.

**2.14.P-We062 Environmental Effects of Individual and Binary Mixtures of Widely Used Drugs and Pesticides in Salmon Aquaculture**

***Davide Asnicar***[1,2] *and Benjamin Patrick de Jourdan*[2]*, (1) University of Padova, Italy, (2) Huntsman Marine Science Center, Canada*

Nearly 75% of the active marine finfish aquaculture sites in Canada use at least one drug or pesticide each year. The Canadian marine finfish aquaculture industry reported use of ten drugs and two pesticides, for the purpose of fish health control or management. The antibiotic drugs are administered in-feed, while the pesticides may be administered in-feed or as a bath treatment. Following the end of a treatment period, in-feed drugs have been detected in sediments where their persistence varies from months to years. Pesticides and therapeutants that enter the marine environment following bath treatments are expected to remain in the water column following release and will disperse and degrade at rates that classify them as non-persistent. Despite their wide usage in Canada, there are still unknowns and concerns regarding the environmental effects of these compounds.

A recent Canadian Science Advisory Secretariat report highlighted two key data gaps, the influence of formulation and the impact of mixtures. The formulation ingredients enhance factors such as solubility, therefore the physical-chemical data derived from the active ingredient may not be appropriate when predicting environmental fate and effects. With multiple aquaculture sites using multiple products of differing fates and modes of action, it is important to understand the synergistic, antagonistic, and additive effects of these multiple stressors. Public concern over these chemicals requires risk assessment on the basis not only of the individual chemical, but also as mixtures.

In this study we performed a series of toxicity tests using the larval stage of the American lobster and the green-sea urchin with the active ingredient and formulated product for 3 commonly used pesticides, SLICE (active ingredient = Emamectin B1a), IVOMEC (Ivermectin) and SALMOSAN 50WP (Azamethiphos) and the antibiotic oxytetracycline. The data from the single compound testing, and available usage data, was used to inform the design of a series of binary mixture trials following a fixed-ratio ray design.

The data generated in this project will provide greater context and understanding for hazards posed by environmental concentrations of these compounds and will serve as valuable input when establishing environmental quality standards or monitoring thresholds.

**2.14.P-We063 Mercury Bioaccumulation in Young-Of-The-Year and Juvenile Sharks in Texas Bays**

***Jasmine Rodriguez***[1]*, Jessica Dutton*[1]*, Faye Grubbs*[2] *and Weston Nowlin*[1]*, (1) Texas State University, (2) Texas Parks and Wildlife Department*

Mercury (Hg) is a global pollutant that occurs at high concentrations in many coastal environments. Mercury contamination is a substantial environmental issue because of its ability to bioaccumulate in organisms and biomagnify in food webs. Effects of Hg exposure on teleost fishes (i.e., developmental, neurological, and cardiovascular) have been documented, but these implications are not fully understood in young-of-the-year (YOY) and juvenile sharks, especially in coastal communities with increased pressure from anthropogenic activity. The purpose of this study was to investigate Hg concentrations in YOYs and juveniles of three coastal shark species [blacktip shark (*Carcharhinus limbatus*), bull shark (*Carcharhinus leucas*), and bonnethead shark (*Sphyrna tiburo*)] in four bay systems (Sabine Lake, Aransas Bay, Corpus Christi Bay, and Lower Laguna Madre) along the Texas portion of the Gulf of Mexico. Sharks (n=30 per species per site) and prey items (n=20 per species per site) were sampled opportunistically via seasonal gill netting from fall 2020 to spring 2022. Shark dorsal muscle tissue and prey items were assessed for Hg concentrations using a direct mercury analyzer (DMA-80), and stable isotopes ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$) were used to assess how the feeding ecology of different shark species and how this influences Hg exposure risk. Hg in putative prey items varied among sites and prey trophic position. For example, red drum (*Sciaenops ocellatus*) had highest Hg in freshwater Sabine Lake, whereas lower trophic level brown shrimp (*Farantepenaeus aztecas*) had higher Hg in intermediate salinity systems. Sharks also showed variation in Hg in response to diet, with larger-bodied and piscivorous bull sharks consistently having grater Hg than bonnetheads, a crustacean consuming specialist. However, there was also substantial variation in shark Hg in response to site location; for example, bull sharks in Sabine Lake had higher Hg than bull sharks in intermediate salinity conditions. Our data fill a critical information gap in Hg concentrations in Texas bay systems for YOY and juvenile sharks as well as for representative species that feed on similar prey items. Additionally, collecting shark species along with putative prey items across broad environmental gradients allows for increased ability to understand larger-scale geographic influences on Hg exposure risk to fishes.

**2.14.P-We064 Effects of Tetrabromobisphenol A (TBBPA) on Downstream Regulatory Element Antagonistic Modulator (DREAM)-regulated Transcription in Adult Zebrafish, Danio rerio**

***Kameron Wong*** *and Erika Holland, California State University, Long Beach*

Changes in intracellular $Ca^{2+}$ concentrations initiate countless cellular responses or signaling cascades including phosphorylation events and gene transcription. The voltage gated $Ca^{2+}$ channels (VGCCs), embedded in the plasma membrane, regulate cellular $Ca^{2+}$ influx. The disruption of VGCCs is associated with many disease states including Timothy syndrome, Parkinson's and Alzheimer's diseases, and type II diabetes. Several environmental pollutants target VGCCs leading to $Ca^{2+}$ signaling disruption (CSD). In particular, the flame retardant tetrabromobisphenol A (TBBPA) is known to inhibit VGCCs, thus causing cyto- and neurodevelopmental toxicity as well as impaired muscular function. While TBBPA can cause CSD, disruptive effects on downstream events are largely unstudied. Of interest to this current study are CSD impacts on $Ca^{2+}$-mediated transcription, specifically of the $Ca^{2+}$-dependent transcription factor DREAM (downstream regulatory element antagonistic modulator). Research on cell lines has confirmed that CSD compounds can alter DREAM-regulated expression; however, impacts in exposed organisms remain uninvestigated. For this study, adult wildtype and DREAMb knockout zebrafish will be exposed to TBBPA, and tissue-specific transcriptional changes will be assessed using RNA-sequencing, and findings will be compared to qPCR data collected on select DREAM-regulated genes. Preliminary research has demonstrated that DREAMb is expressed in the heart and brain of adult zebrafish, and we are currently determining DREAMb expression in pancreatic tissue. This research will further our understanding of CSD chemical impacts and contribute to the development of tools available for monitoring the sublethal effects of CSD pollutants exposure. The wide tissue distribution of DREAM suggests that CSD could have vast impacts on neuronal, muscle, and endocrine health.

**2.14.P-We066 Increased Popularity in Liquid Resin 3D Printing with Poor Understanding of Feedstock Hazard**

***Mark Ballentine****, Alan Kennedy, Nicolas Melby, Anthony Bednar, Robert Moser, Lee Moore, Erik Alberts, Charles Laber and Rebecca Crouch, U. S. Army Corps of Engineers*

The availability and popularity of additive manufacturing (AM) has increased over the past decade. Environmental hazard assessment and safety data sheets for 3D printer feedstocks has lagged technology development. While the majority of environmental studies have focused on fused filament fabrication printing, vat photopolymerization, otherwise known as stereolithography, may have unique risks relative to other AM technologies due to mishandling of uncured monomers/oligomer feedstocks. Vat polymerization AM products have recently substantially reduced in cost and its decreasing cost enabling increased residential use. The lack of publicly available chemical data for six uncured liquid resins was explored through method development. The commercially available vat photopolymerization liquid resins were extracted with methylene chloride for component identification and quantitation via gas chromatography coupled with mass spectrometry (GC-MS) or flame ionization detector (GC-FID). The acute and chronic toxicity of the six uncured resins to *Ceriodaphnia dubia* was explored. Nominal values for two-day median acute toxicity (LC50) values ranged from 2.6 – 38 mg/L. Inhibition concentrations causing 25% reduction in reproduction (IC25 values) ranged from 0.33 – 16 mg/L. Since many non-commercial users likely do not understand the potential hazard of uncured monomers in liquid resins, these data were generated to provide some knowledge of the relative toxicity of these materials so consumers can develop a plan for the use, handling, and waste management of uncured vat photopolymerization resins.

**2.14.P-We067 Assessing Gene and Protein Expression in Mucus of Fathead Minnows Exposed to Graded Nutrient Loadings in Contained Model Wetlands**

***Krista Robertson***[1]*, Nicholas Blandford*[1]*, Leah Dickenson*[1]*, Kenneth Jeffries*[1]*, Denina Simmons*[2]*, Markus Hecker*[3]*, Bruce Hardy*[4] *and Vince Palace*[5]*, (1) University of Manitoba, Canada, (2) Ontario Tech University, Canada, (3) University of Saskatchewan, Canada, (4) Myera Group, Canada, (5) IISD Experimental Lakes Area (IISD-ELA), Canada*

Wild rice (*Zizania palustris)* contains high concentrations of nitrogen and therefore generates a high yield when using nitrogen rich, ammonium based fertilizers. Fish waste (FW) as fertilizer is a sustainable method of adding appropriate levels of nitrogen and phosphorus to allow optimal plant growth while recycling waste from finfish aquaculture operations. However, introducing nutrients to an aquatic ecosystem presents risks associated with elevated ammonia and nitrite with the potential for toxic concentrations. In addition, phosphorus in the FW can alter the trophic state of receiving waters potentially producing toxic algal blooms and creating an oxygen depleted ecosystem. We assessed gene and protein expression pathways in fathead minnows (*Pimephales promelas*) exposed to incremental additions of FW for 140 days in wetland mesocosms seeded with wild rice. Stress gene expression pathways were assessed using a quantitative polymerase chain reaction (qPCR) array, the EcoToxChip, in dermal mucus and liver tissue. Proteomic analysis of mucus was also performed to assess differential protein expression among fatheads exposed to the graded nutrient additions. This study will further develop the use of mucus genetic and proteomic analysis to limit lethal sampling methods in the field, and help mitigate environmental risks associated with adding FW to aquatic ecosystems.

**2.14.P-We068 Assessing the Effect of Aquaculture Waste on Aquatic Invertebrate Communities in Mesocosms Planted with Wild Rice**

***Leah Dickenson***[1]*, Vince Palace*[2]*, Mark L. Hanson*[1]*, Nicholas Blandford*[1]*, Krista Robertson*[1]*, Lisa Peters*[2] *and Bruce Hardy*[3]*, (1) University of Manitoba, Canada, (2 )IISD Experimental Lakes Area (IISD-ELA), Canada, (3) Myera Group, Canada*

The waste products produced from aquaculture operations have the potential to be used as sustainable replacements to conventional fertilizers for northern wild rice (*Zizania palustris*) cultivation. However, the addition of waste into a flooded wild rice landscape could have serious implications on the aquatic environment including depleted dissolved oxygen concentrations, elevated nutrient concentrations (i.e., ammonia and phosphate), suspended solids, and turbidity levels. These deleterious conditions could secondarily affect the biological components of the aquatic ecosystem, including resident fish and invertebrate communities. We examined the effects of adding varying amounts of aquaculture waste to mesocosms planted with wild rice on the abundance and community structure of aquatic invertebrates. Circular, flatbottomed polyethylene mesocosms (2 m diameter; ~3200 L) were amended with 30 cm of soil and 40 cm of water. Wild rice seed was planted in the fall of 2021. After the seed germinated in each mesocosm, zooplankton, benthic invertebrates, and fat head minnows (*Pimephales promelas*) were added to the mesocosms from a donor site. The mesocosms were then treated with a gradient of waste from an aquaculture hatchery on a bi-weekly basis. To identify aquatic invertebrate community composition, zooplankton were sampled prior to every nutrient addition using activity traps and benthic invertebrates were sampled from a sediment collection at the end of the study. Then traditional enumeration methods were conducted. This study provides an opportunity to examine nutrient uptake by wild rice and threshold rates of fertilization that result in negative impacts to water quality and aquatic invertebrate communities. The information gathered from this study will be used to determine the most suitable concentrations of aquaculture waste for application to larger scale flooded wild rice patty systems, so that ambient water quality conditions can be maintained.

**2.14.P-We069 Behavioral and Transcriptomic Effects of Paroxetine on the Fathead Minnow (Pimephales promales)**

***Meagan Elizabeth Bell****, Daniel J. Sullivan and Weichun Huang, U.S. Environmental Protection Agency*

Paroxetine (PXT) is an antidepressant serotonin selective reuptake inhibitor (SSRI) and has been frequently detected in surface waters and other environmental matrices in North America. Previous research has indicated the presence of PXT in tissue of fauna inhabiting affected freshwater ecosystems, but little is known of how PXT may influence transcriptional and behavioral response of fish. The fathead minnow (*Pimephales promelas*, FHM) is a broadly distributed and well-studied small, freshwater fish species, thus making it a good candidate to use as a model for investigating the effects of chemicals in the freshwater environment. PXT alters neurotransmission through inhibiting reabsorption of serotonin in neurons, allowing increased presence of serotonin in signals between nerve cells, therefore we hypothesized that increased levels of serotonin in FHM through PXT exposure would influence behavior and gene expression. Prior research on human consumption of PXT and other SSRIs, has shown that increased serotonin levels may cause side effects of fatigue, dizziness, and slow reaction to stimulants. Because of these side effects, we predicted that increased concentrations of PXT would cause decreased movement in response to photoperiodicity because of increased serotonin levels. Likewise, we predicted that differential gene expression would occur at greater rates with increasing concentrations of PXT. To evaluate how PXT influences gene expression and behavior of FHM, we exposed larval FHM (5 days post fertilization) for 24 hours to ten concentrations of PXT. Larval FHM from all treatments were homogenized to isolate RNA and generate mRNA libraries for sequencing. Concentrations used in evaluation of gene expression ranged from 1.5mg/L to 50ng/L and moderately hard reconstituted water (MHRW) as a control. Behavior assays were performed using larval FHM exposed to control and highest three concentrations (1.5mg/L, 0.5mg/L, and 0.15mg/L) of PXT and were analyzed to evaluate how individuals responded to photoperiodicity. Here, we will present the results of our research and the importance of using unique biological endpoints to evaluate the effects of chemicals that may not be detected using traditional endpoints (mortality, reproduction, growth). Further, we will discuss our findings on how PXT and mechanistically similar SSRIs may have an adverse effect on fauna inhabiting freshwater systems where PXT and SSRIs are found.

**2.14.P-We070 Temperature Modulation of Acute Lethal Toxicity of Individual Polyaromatic Compounds to Marine Organisms**

*Hannah Jones[1], Danielle A. Philibert[2], Benjamin Patrick de Jourdan[2] and* ***Christopher J. Kennedy****[1], (1) Simon Fraser University, Canada, (2) Huntsman Marine Science Center, Canada*

Understanding and predicting the effects and risks of an oil spill to marine organisms requires information on the toxicity of whole oil as a complex mixture and the individual components of oil such as the polycyclic aromatic compounds (PACs). Acute (1- to 48-h exposure) lethal bioassays (for the determination of various toxicological parameters (e.g. LC10, LC50, NOAEC, LOAEC) were conducted for 10 polyaromatic compounds on a range of marine organisms including unicellular and macro-algae, invertebrates and vertebrates. Appropriate PAC concentrations were maintained using headspace-free exposure vessels and a passive dosing (PD) chemical delivery system. The specialized exposure systems for PD included 20 ml small volume, static vessels as well as 300 ml, flow-through vessels that delivered well-controlled, solvent-free exposures in toxicity tests. PACs and heterocycles were introduced to silicone O-rings, which were added to seawater. The loaded polymer served as a reservoir to buffer chemical losses based on partitioning from the polymer to seawater. Toxicity tests were performed under 3 temperature regimes (5-21°C, depending on the test organism). Results indicate that 1) there is some consistency to the toxicity ranking of the tested PACs regardless of the organism tested, 2) algae are consistently the least sensitive to PACs, followed by fish and then invertebrates, 3) early life stages are generally more susceptible to PACs compared to older stages, 4) increasing temperature increases the toxicity of most PACs (not all changes are proportional and are PAC-specific) to all organisms tested. The data provided by these studies will aid in the development of oil spill models, risk assessment, oil spill responses, and oil spill monitoring plans for managing marine organisms in the event of potential spills in marine coastal waters.

**2.14.P-We071 Recovery of Benthic Macroinvertebrate Assemblages in Association with Upgrades to Wastewater Treatment Plants**

***Sean McLay*[1]*, Adam G. Yates*[1]*, Dominika Celmer-Repin*[2]*, Keegan Hicks*[3]*, Edward M. Krynak*[1]*, Hadi Dhiyebi*[1]*, Leslie Bragg*[1] *and Mark R. Servos*[1]*, (1) University of Waterloo, Canada, (2) Regional Municipality of Waterloo, Canada, (3) Alberta Environment and Parks, Canada*

Treated effluent from wastewater treatment plants (WWTPs) can be a source of substantial nutrient and contaminant loading, altering water chemistry and ecological conditions in receiving waters. To meet new regulations and increasing demand, the Region of Waterloo invested in major upgrades to the Kitchener and Waterloo WWTPs, the two largest WWTPs in the Region, over the past decade. As a part of efforts to monitor the effectiveness of these investments, the Region of Waterloo initiated a benthic macroinvertebrates (BMI) monitoring program sampling upstream and downstream of effluent outfalls. Using data collected in the fall every three years between 2009 to 2018, the response of the BMI assemblage to upgrades at the Waterloo and Kitchener WWTPs was assessed. Spatial and temporal analyses of BMI suggested that the impacts of the Waterloo and Kitchener WWTPs are reducing over time, with upstream and downstream assemblages becoming more similar following upgrades. These findings support that WWTP upgrades are succeeding in reducing the influence of effluent discharged into the receiving waters. With some upgrades to WWTPs occurring within a year prior to the last sampling year (2018), and delays in response to amelioration of stressors sometimes observed in BMI assemblages, recovery is likely still ongoing. Continued monitoring is therefore recommended to capture potential further recovery.

**2.14.P-We072 Global Occurrence and Hazards of Antipsychotic Pharmaceuticals in Influent Wastewater, Effluent Discharges and Surface Waters**

***Adam Wronski*** *and Bryan W. Brooks, Baylor University*

Despite the global prevalence of pharmaceuticals in surface waters, sublethal aquatic toxicology information is limited or nonexistent for many of these contaminants, particularly of chronic responses plausibly linked to molecular initiation events that appear largely conserved across vertebrates. To better understand the prevalence of these compounds, we conducted a critical review of the available refereed literature regarding the occurrence of 21 antipsychotics in wastewater effluent and surface waters. Because the majority of sewage remains poorly treated or untreated around the world, we also examined occurrence in wastewater influent. When sufficient information was available, we developed probabilistic environmental exposure distributions (EEDs) for each compound in each matrix by geographic region. We then performed probabilistic environmental hazard assessments (PEHA) using therapeutic hazard values (THVs) of each compound, due to limited sublethal aquatic ecotoxicology information for this class of pharmaceuticals. From these PEHAs, we determined predicted exceedances of the respective THVs for each chemical among matrices and regions. We then compared THV values for antipsychotics to other human medications and noted that THV values for these contaminants are typically much lower than other groups of pharmaceuticals known to adversely affect aquatic life. In wastewater effluent discharges and surface waters, sulpiride was the most detected antipsychotic, which may, in part, be influenced by the minimal human metabolism of this compound. However, THV percent exceedances were minimal (0.3%) for this compound. In contrast, we observed relatively high exceedances of THVs for chlorpromazine (44.2%), haloperidol (48.4%), and perphenazine (78.3%) in effluents and for chlorprothixene (35.4%) and flupentixol (98.8%) in surface waters. Further, we specifically identified elevated aquatic hazards for relatively understudied antipsychotics, which highlight important data gaps that can inform future environmental toxicology and chemistry research efforts.

**2.14.P-We075 Fluoxetine alters behavior and increases predation risk for an aquatic snail**

***Christopher G. Goodchild*** *and William G. Owens, University of Central Oklahoma*

The selective serotonin re-uptake inhibitor fluoxetine has been detected in wastewater treatment facility effluent discharged into freshwater ecosystems. There is evidence that aquatic organisms exposed to fluoxetine exhibit altered behavior, but whether fluoxetine affects survival remains unclear. One potential route for fluoxetine to reduce survival is by altering antipredator behavior. The aquatic snail species *Physa acuta* is an applicable model organism for this line of inquiry as it has well-characterized antipredator behaviors (e.g., retraction into shell, decreased activity, migration to the water's surface). To test whether fluoxetine increases risk of predation, we exposed snails to 5, 25, 125 ug/L fluoxetine or deionized water (control) for 8 days. Using this exposure design, we conducted two experiments. In the first experiment, we placed fluoxetine-exposed snails in a mesocosm with a crayfish and measured survival over 24 hours. In the second experiment, we measured variation in antipredator behavior after fluoxetine exposure. Snails exposed to fluoxetine had reduced survival in the crayfish mesocosm compared to controls. Additionally, fluoxetine exposure increased righting time and decreased migration to the water's surface and activity. These data suggest fluoxetine may indirectly affect survival by increasing predation risk.

**2.14.P-We076 Halogenated PAHs can activate the AHR2 of fishes**

***Jon Doering***[1]*, Justin Dubiel*[2]*, Hunter Johnson*[2]*, Zhe Xia*[3]*, Gregg Tomy*[3] *and Steve Wiseman*[2]*, (1) Louisiana State University, (2) University of Lethbridge, Canada, (3) University of Manitoba, Canada*

Polycyclic aromatic hydrocarbons (PAHs) are a diverse class of compounds characterized by the presence of two or more fused aromatic rings. PAHs are naturally occurring, but the extraction and utilization of fossil fuels can increase loading into the environment and cause adverse effects in wildlife. Most research on environmental concentrations of PAHs and their toxicities in wildlife have focused on 16 PAHs that were identified as priority pollutants by the U.S. Environmental Protection Agency (USEPA). However, other PAHs have received less attention. Halogenated PAHs (HPAHs) with one or more bromine or chlorine substituent attached have been detected in aquatic environments and in tissues of fishes. HPAHs closely resemble polychlorinated dibenzo-p-dioxins/dibenzofurans (PCDD/Fs) and polychlorinated biphenyls (PCBs). Certain PCDD/Fs and PCBs are collectively known as dioxin-like chemicals because they act as agonists of the aryl hydrocarbon receptor 2 isoform (AHR2) of fish causing dysregulation in receptor function which can lead to a variety of toxicities. However, whether HPAHs are agonists of the AHR2 of fish was unknown. To address this question, an in vitro luciferase reporter gene assay was performed using COS-7 cells transfected with the AHR2 of zebrafish (Danio rerio). The potency for activation of AHR2 of zebrafish was tested for 11 brominated, chlorinated, and mixed-halogenated anthracenes. Potencies of each halogenated anthracene were compared to potencies for the prototypical dioxin-like chemical, 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), and to the parent anthracene. Anthracene did not activation AHR2 of zebrafish up to 10,000 nM, but 8 of the 11 halogenated anthracenes caused activation. In general, potency for activation increased with increasing halogenation. The 3 halogenated anthracenes that did not activate the AHR2 of zebrafish had either 1 or 2 halogens, while the most potent chemicals had 3 or 4 halogens. Among the 8 halogenated anthracenes that activated the AHR2 of zebrafish, potencies relative to TCDD ranged from 0.0001 to 0.1. Results of this study demonstrate that HPAHs have the potential to activate the AHR2 of fishes with potencies comparable to dioxin-like PCBs. Therefore, HPAHs in the environment could represent an ecological risk to fishes and warrant further investigation.

**2.14.P-We077 Polyisocyanate Prepolymers: Investigation of Structure-Property Relationships to Aquatic Exposure and Acute Aquatic Toxicity**

*Steve Seneker*[1]*, Glenn Simon*[2]*, Stephanie Snyder*[3]*, Mark Spence*[4] *and* ***Robert J. West***[5]*, (1) Anderson Development Company (retired), (2) Simon Toxicology, LLC, (3) Covestro LLC, (4) International Isocyanate Institute, Inc., (5) Tessenderlo Kerley, Inc. / NovaSource*

The potential for exposure and associated health and environmental hazards of diisocyanate (DII) monomer substances can be of concern during manufacture or application of polyurethane (PU) materials. These exposure and hazard potentials can be reduced by reacting DII monomers onto the terminal hydroxyl groups of various polymeric polyether and polyester polyols to produce isocyanate-terminated prepolymers. The performance of

PU materials produced from these prepolymers can be precisely tailored through selection of their precursors from a wide variety of DII monomers and polyols. Hydrolysis of DII and the isocyanate prepolymer substances is suspected to lead to formation of cationic ($R\text{-}NH_3^+$ terminated) polymers in the environment, and this is a basis for their presumed high aquatic hazard potential. This work explored how variation in structures of the DII and polyol building blocks and in the physical–chemical properties of isocyanate prepolymers contributes to their aquatic exposure and hazard potentials. A group of 17 prepolymers ranging in molecular weight (MW) from 750 – 6,600 g/mol, functionality from 2 – 4, and calculated log $K_{ow}$ from -5 to +45 were synthesized from among four different DII monomers and nine polyol types. The aquatic exposure potentials were determined using a variation of OECD Guideline 120, from which the water-accommodated fractions (WAF) were obtained for testing acute immobilization of *Daphnia magna* according to OECD Guideline 202. For prepolymer loading rates of both 100 and 1000 mg/L in water, water-extractable reaction products (measured as dissolved organic carbon, DOC) were not quantifiable where the calculated log $K_{ow}$ value of the prepolymer was > 10. Otherwise, the quantifiable DOC concentrations exhibited across this substance class showed an expected inverse correlation with calculated log $K_{ow}$. All 17 prepolymer substances exhibited 48-h median effective loading rates ($EL_{50}$) of > 100 mg/L, and 16 of 17 exhibited $EL_{50}$ > 1,000 mg/L. Thus, the acute aquatic hazard potential for this substance class is very low, regardless of structure features, physical-chemical properties, or magnitude and composition of their WAF. The revelation of these trends in aquatic exposure and hazard potentials could now provide a basis for regulatory screening of existing or new isocyanate prepolymer substances, while also informing the design of such substances having reduced exposure and/or hazard profiles.

**2.14.P-We078 Examining the Impact of Pyrethroid Leaching From Insecticide Treated Net Fishing on Oxidative Stress Biomarkers in P. Promelas**

***Deirdre Honoria Doyle Love***[1], *David Larsen*[2], *Joseph H. Bisesi Jr.*[1] *and Melissa Filgueiras*[1], *(1) University of Florida, (2) Syracuse University*

The distribution of insecticide treated mosquito nets (ITNs) is a great public health success story, with studies suggesting these nets reduced the global burden of Malaria by approximately 40%. ITNs are typically treated with pyrethroids, which are known to have a low toxicity to mammals and high toxicity to aquatic organisms. Recent studies have shown that ITNs are being used for alternative practices, including fishing. In a study from a waterside community located on Lake Tanganyika, 87.2% of people utilized their mosquito net for fishing as opposed to malaria protection. Coinciding with reports of off label fishing, there are reports of a decrease in fish quality and quantity. Our previous research has shown that pyrethroids from ITNs can rapidly leach into water, causing overt toxicity in both larval fish and Daphnia. Another cause of concern from pyrethroid exposure is oxidative stress from reactive oxygen species (ROS) for a variety of aquatic organisms. The goal of this experiment is to determine whether or not ITNs exposure will lead to oxidative stress for *P. promelas* larvae following a sub-lethal exposure. Organisms will be exposed to 1cm$^2$ and 5cm$^2$ sized nets in order to determine the appropriate sub-lethal concentration. Following this determination, organisms will be exposed to the sub-lethal concentration for 7 days and at the end of the experiment, we will run a DCFDA ROS Assay. Fish samples will be washed in de-chlorinated tap water and then homogenized in 120mL of cold PBS, and centrifuged at 15,000g for 20 minutes at 4°C. 100mL of supernatant will be removed and diluted in warmed PBS, and added to a 96 well plate. CM-$H_2$DCFDA will be added to reach a final concentration of 1mM and then the plate will be incubated at 37°C for 30 minutes. Our plate will then be added to the microplate reader with excitation (485nm) and emission (520nm). We will also measure Superoxide Dismutase (SOD) and Catalase (CAT) expression using qPCR in the *P. promelas* larvae to expand our understanding of oxidative stress. We expect to see an increase in ROS, CAT and SOD activity due to the induction of oxidative stress by pyrethroid compounds. Results from this study are expected to increase our understanding of the potential for sublethal effects from ITN fishing.

**2.14.P-We079 Water quality and elemental distribution in Typha domingensis in Cienega of Tamasopo Natural Wetland, Mexico**

***Cynthia Wong Arguelles***[1]*, Daniel Cruz Hernandez*[2] *and Candy Carranza Alvarez*[3]*, (1) Tecnologico Nacional de Mexico campus Ciudad Valles, Mexico, (2) Universidad de Chiapas, Mexico, (3) Universidad Autonoma de San Luis Potosi, Mexico*
Wetlands represent a unique hydrological feature of the landscape, as well as being a source of water supply. Therefore, its quality is directly related to the life and economy of the inhabitants of its surroundings. The purpose of this work was to evaluate the water quality of the springs of the natural wetland "Ciénega de Tamasopo", which are the main source of water available for human use among the population living near this wetland. The physicochemical and biological parameters of these springs have not been recently reported. Some *in situ* parameters (temperature, pH, total dissolved solids, and turbidity) were analyzed in the water samples from the springs before and after the rainy season. The results showed that the temperature in the springs oscillates between 22 and 25 ° C and the pH varies between 7.2 and 8.2. The total water hardness values ranged from 229 to 1030.67 mg/L $CaCO_3$, from 28.74 to 302.30 mg/L sulfates ($SO_4^{2-}$), and from 0.21 to 1.01 mg/L nitrates ($NO_3$). The total hardness and sulfate concentration exceeded the maximum permitted limits stipulated in the regulations (before the rainy season), it is suggested to carry out frequent monitoring in these springs, in order to guarantee their quality and define the type of use. On the other hand, to evaluate the phytoextraction of the elements by the *Typha domingensis* plants present inside the wetland, adult plants of this plant species were collected at five sampling sites. Fresh root, leaf, and seeds of *Typha domingensis* were collected from the study site. These samples were analyzed with synchrotron light at the Canadian Light Source in Saskatoon, Sasketchewan, Canada. The analysis with µ-XRF using synchrotron light in the VESPERS line was used to investigate the distribution of macronutrients (Ca, K, S, and P) and micronutrients (Cu, Fe, Mn, Zn, and Cl) in root, leaf, and seeds of *Typha domingensis* collected in the "Ciénega de Tamasopo" natural wetland. The results showed that these elements were absorbed and accumulated in the different tissues of the plant. This suggests that processes of absorption, distribution, and accumulation of these elements occurred in various plant tissues.

**2.14.P-We080 The Effects of Polyethylene Microbeads on Crayfish-Annelid Symbiosis**

***Cameron Bryce Braswell***[1]*, Tyler Allen*[2]*, Robert Creed*[3]*, Bryan Brown*[2] *and Austin Gray*[2]*, (1) Virginia Tech, (2) Virginia Polytechnic University, (3) Appalachian State University*
According to the EPA, annually within the United States, an estimated 5.5 million tons of plastic waste evades waste management practices and finds itself scattered throughout the environment. Microplastic pollution in marine and freshwater systems has become a major environmental issue where particle concentrations can range from (4,137 to 12,000 items/m-3). This poses the question - what impact does microplastic pollution have on species interactions in freshwater ecosystems? Through the use of exposure-response assays, we've investigated whether polyethylene microbeads (63-75um) at a concentration of (25mg/L) affected crayfish and their symbiotic annelids (Branchiobdellidans). Following a 96-hour exposure, we observed mortality and growth of both taxa for 2 months. At the conclusion of the experiment, crayfish and annelids demonstrated resilience to microplastic exposure with mortality ranging between (0 to 21%), respectively. There were no differences observed in crayfish growth rate between control and experimental treatments ($p=0.4667$). Average growth of crayfish from control treatments ranged between (Mean ± SE) (3.3 ± 1.93). Average growth of crayfish from experimental treatments ranged between (Mean ± SE) (18.15 ± 24.09). Although microbeads had no toxicological effects to crayfish, there were apparent acute toxicological effects to their symbionts. Crayfish mortality and growth were not influenced by microbeads, which warrants further discussion into the effects of differing particle morphologies such as plastic fibers and fragments.

**2.14.P-We081 A Modernized Protocol to Assess the Aquatic Toxicity of Oil**

***Benjamin Patrick de Jourdan***[1]*, Jennifer Loughery*[2]*, Gina Coelho*[3] *and Kenneth Lee*[4]*, (1) Huntsman Marine Science Center, Canada, (2) Hemmera, Canada, (3) Bureau of Safety and Environmental Enforcement, (4) Fisheries and Oceans Canada, Canada*
The Chemical Response to Oil Spills: Ecological Effects Research Forum (CROSERF) created a standardized protocol for comparing the toxicity of physically dispersed oil to chemically dispersed oil to investigate

dispersant toxicity and assist oil spill responders in the early 2000s. Since then, many revisions have been made to the original protocol to diversify the intended use of the data generated, adapt to newer technology, and to adapt to non-conventional oils and fuels. Under the Multi-Partner Research Initiative (MPRI) for oil spill research under Canada's Oceans Protection Plan (OPP), a network of 45 participants from seven countries representing government, industry, non-profit, private, and academic sectors was established to identify the current state of the science and formulate a series of recommendations to modernize the oil toxicity testing framework. The participants formed a series of working groups, targeting specific aspects of oil toxicity testing, including experimental conduct, media preparation, phototoxicity, analytical chemistry, reporting and communicating results, interpreting toxicity results, and use of toxicity data to inform oil spill effects models. Recognizing that a single protocol would not be able to address the multitude of questions currently asked; working group participants reached a consensus that a modernized protocol to assess the aquatic toxicity of oil should be based on a 'fit-for-purpose' manner, where the methods and approaches are driven by the specific objectives of the study.

This presentation will highlight some of the key recommendations from the working groups and provide guidance for practitioners to ensure their data is reproducible, reliable, and has utility for informing oil spill response decisions.

**2.14.P-We082 The Impact of Final Brood Release on Terminal Dry Weight in Daphnia Life Cycle Studies**

*Amanda Milligan[1], Gunther Ulric du Hoffmann[1], Timothy Springer[2] and* ***Sean Gallagher[1]****, (1) Eurofins Agroscience Services, (2) Springer Ecotox Consulting, Canada*

The Daphnid Chronic Toxicity Test (U.S. EPA OCSPP 850.1300 (October 2016); OECD 211 (October 2012)) is required for the registration of crop protection products in the U.S. and for the phase II environmental risk assessment of human medicinal products in the EU. The daphnid reproduction study also provides chronic ecotoxicity data for shipping classifications and labelling requirements. The endpoints in the study used for a statistical evaluation of the no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) are survival, reproduction and growth. While OECD 211 recommends growth measurements (e.g. length of parental animals), OCSPP 850.1300 indicates growth of the $F_0$ adults should be determined at test termination and further indicates that both dry weight and length measurements are preferred. Statistically significant impacts on growth are frequently used to determine NOEC and LOEC endpoints in daphnid chronic toxicity tests.

Anecdotal observations from chronic daphnid studies suggest that the timing of release and the size of the final brood produced by *Daphnia magna* may impact the dry weight measurement for individual daphnia. The hypothesis: females that release their final brood on or nearer to the day of test termination will have empty brood pouches and will have lower dry weight on average than females that do not release a brood on or near the day of test termination.

Historical control data from 40 semi-static, *Daphnia magna* chronic toxicity studies conducted over an approximately 5-year period were collated to create a database including day of final brood release, number of young in the final brood, along with length and dry weight of adults at termination. Parental generation exposures lasted 21 days with initiation of neonate production typically occurring on Day 7 or 8 of the test. The number of young produced per surviving female averaged 200. Statistical evaluations were performed to examine possible correlations between timing and size of the final brood produced and terminal growth measurements, particularly dry weight, in *Daphnia magna* reproduction studies.

This poster will provide an overview of the aggregated historical control data from the toxicity study reports, results of statistical evaluation of correlations between reproduction and terminal growth measurements.

## 2.14.V Poster Only: Aquatic Toxicology, Ecology and Stress Response

### 2.14.V-02 Evaluation of the effect of humic acid on inorganic chemicals using Daphnia magna

***Tatsuo Abe***[1], *Shinko Ito*[1] *and Yu Tachibana*[2], *(1) Tsuruoka College, Japan, (2) Nagaoka University of Technology, Japan*

Humus substances derive from soil organic matter. Dissolved organic matter is fine particle (less than 100 ~ 1000 nm) in water. Natural water contains humus substances derived from organisms, which are a mixture of a wide variety organic substances and a complex of high molecular- weight compounds. Humus substances have structural diversity and various physicochemical properties: colloidal, surface-active, dispersion, and chelate. These properties might affect inorganic chemicals.

In this study, humic acid was used as humus substance. Several organic acids as degradation products of humic acid and sodium salt compounds as inorganic chemicals were used to evaluate combined effect of humic acid.

*Daphnia magna* acute immobilization test was used for this study. The test method was based on OECD Test Guideline 202. Each 100 mL test solution in beaker was prepared in M4 medium. 20 neonates (daphnia within 24 hours old) placed each concentration and control, each beaker observed after 24 and 48 hours to obtain a 50% effective concentration ($EC_{50}$). Test reagent used humic acid (FUJIFILM Wako Pure Chemical, Japan), organic acids, and sodium salts, each of which was prepared as concentrated solution in M4 medium. The test conducted with control, humic acid control and five concentrations, and 2.5 mg of humic acid was added to all concentration and humic acid control.

Formic acid, succinic acid, and malic acid indicated almost same $EC_{50}$s with or without humic acid. The effect of oxalic acid dihydrate with humic acid was lower than without humic acid. Sodium salts also indicated almost same $EC_{50}$s with or without humic acid. In sodium salts containing sulfur, the effects with humic acid tend to be lower than without humic acid.

### 2.14.V-04 Effects of phenanthrene exposure on the B-esterases, antioxidant enzymes activities and oxidative damage in Octopus maya (Voss and Solís Ramírez, 1966) embryos

*Letícia Aguilar, **Gabriela Rodríguez-Fuentes**, Carlos Rosas-Vázquez, Claudia Caamal-Monsreal, Elsa Noreña-Barroso, Gissela Moreno Ortiz and María Gómez-Maldonado, National Autonomous University of Mexico (UNAM), Mexico*

Little is known about the impact of pollutants on cephalopods, important ecological and commercial animals. This study investigated the effect of phenanthrene (Phe) in *Octopus maya* embryos by the activities of the B-esterases: Acetylcholinesterase (AChE), and Carboxylesterases (CbE), and on the antioxidant defense system: superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST) and total glutathione (GSH), and on the oxidative damage indicators: protein carboxylation (PO) and lipoperoxidation (LPO). Octopus embryos were exposed to concentrations of 10 and 100 µg/L of Phe. The activities of AChE, CbE, SOD, CAT and GST and the concentration of GSH, PO and LPO were measured at 7, 14, 20, 26, 32, and 36 days of exposure. Embryo development was divided into organogenesis (stages X-XIII; sampling 7), activation (when heart starts beating, stages XIV-XVI; samplings 14, 20 and 26) and growth (stages XVII-XIX; samplings 32 and 36). AChE activity significantly decreased at the sampling day 36 (growth phase) in the concentration of 100 µg/L of Phe. CbE activity significantly decreased on the sampling days 20, 26, and 36 (organogenesis and growth phases) for both concentrations (10 and 100 µg/L). Oxidative stress caused by Phe in *O. maya* embryos was evidenced by the induction of SOD, CAT and GST and an increase of the levels of GSH and PO, mainly at the activation (sampling day 26), when the circulatory system is fully functional. Our work indicates that CbE is a sensitive biomarker of exposure to Phe in *O. maya* embryos. Phe generates oxidative stress and oxidative damage in this species, thus if present, Phe could create an impact on the populations of these mollusks that are endemic and restricted to the Gulf of Mexico and the Mexican Caribbean region.

## 2.15.P Late Breaking Science: Aquatic Toxicology, Ecology and Stress Response

### 2.15.P-Th151 Metal Accumulation in the Shell and Soft Tissues of Chinese Mysterysnails

***Amanda Mullen***[1]*, Lynne Beaty*[2]*, Adam Martin Simpson*[2] *and Sam Nutile*[2]*, (1) Penn State University - Erie, (2) Penn State Behrend*

Invasive species threaten non-native habitats by outcompeting native species, disrupting food chains, and reducing biodiversity. The Chinese mysterysnail (*Cipangopaludina chinensis*) was introduced to the Great Lakes region in the 1930s and 1940s and is now considered an invasive species. In addition to the typical adverse effects caused by invasive species, mysterysnails also accumulate toxic metals within their bodies that can affect native ecosystems into which they have invaded. As a self-preservation mechanism, snails may transfer most acquired metals into their shells away from important organs, but relative allocation of metals to shells versus various soft tissues is unknown. Therefore, this study aims to determine how the concentrations of metals differ between the shell and soft tissues of the Chinese mysterysnail. To determine the distribution of metals within these snails, snails, water, and sediment samples were collected at documented invasion sites — Presque Isle State Park, Pymatuning Reservoir, Lake Canadohta, and Lake Pleasant — in 2021 and 2022. Collected snails were dissected to separate the shell and internal organs (gonad, mantle, foot, gut) and metal concentrations were compared to concentrations in water and sediment. Metals allocated to specific tissues differently depending on the metal. For example, cadmium and zinc were highest in the gut, while iron, lead, and manganese were most concentrated in the operculum. Shells had relatively low metal accumulation, which was surprising considering the calciferous nature of the snail shells. Biota-sediment accumulation factors (BSAFs) and bioconcentration factors (BCFs) calculated using sediment and water concentrations, respectively, revealed the majority of metals were accumulated from water, suggesting invasive snails rely predominantly on filter feeding to obtain energy in their non-native habitats. Understanding the concentration of metals in the different tissues of mysterysnails will help understand how their physiology influences their ability to respond to contamination and what additional threats they pose to native species. This information can aid in developing management plans for mysterysnails in the Great Lakes region, especially regarding the species' ability to accumulate and sequester toxic metals.

### 2.15.P-Th152 Application of the Adverse Outcome Pathway Construct to Determine Nanogold Toxicity to Freshwater Organisms

***Victor Wepener****, Avela Mbangatha, Suanne Bosch and Tarryn Botha, North-West University, South Africa*

The adverse outcomes pathway (AOP) is a conceptual framework that can be used to show the linkages between sub-cellular initiating events and adverse outcomes. The AOP framework integrates (eco)toxicological processes across levels of biological to be implemented in studies from understanding stressor mediated adverse effects to management to ecological risk assessment. The AOP is a modular framework that elucidates the causal relationships between sub cellular processes (Molecular Initiating Events); cellular, organ level, organ system and organism-level processes (Key Events) and population and ecosystem community responses (Adverse Outcomes). In this presentation we demonstrate the application of the AOP concept using the standard macroinvertebrate and fish test organisms *Daphnia magna* and *Danio rerio* that were exposed to nanogold and its ionic equivalent. Responses at different levels of biological organisation from metabolomics to functional responses (species interactions) and reproduction were evaluated. The results indicate that the responses are not metal (i.e. gold) specific but rather ionic and particle specific. In addition, there were species specific responses, which does not allow for a read-across species approach. These data indicate that using the traditional metal-based responses to evaluate nanogold particles would be inappropriate as tool for risk assessment of nanomaterials.

### 2.15.P-Th153 Development of molecular indicators for municipal effluent exposure responses in longnose dace (Rhinichthys cataractae) caged in artificial streams

***Patricija Marjan***[1]*, Christopher Martyniuk*[2]*, Maricor Jane Arlos*[3]*, Mark R. Servos*[4]*, Norma Ruecker*[5] *and Kelly*

*Munkittrick[1], (1) University of Calgary, Canada, (2) University of Florida, (3) Department of Civil and Environmental Engineering, Canada, (4) University of Waterloo, Canada, (5) City of Calgary, Canada*
Transcriptome profiling can reveal initial biological responses to environmental signals, including environmental substances of concern (ESOCs) in municipal wastewater effluents (MWWEs). However, identifying candidate gene markers that characterize complex mixtures such as MWWEs remains challenging. We determined transcriptomic responses in longnose dace (*Rhinichthys cataractae*; LND) exposed to 5% final MWWE over time to uncover molecular signals to be used as reliable indicators of tertiary MWWE exposure. The Advancing Canadian Water Assets facility located within the Pine Creek municipal wastewater plant (Calgary, AB) has 12 x 340 m raceways receiving treated MWWE or water from the Bow River. LND collected from an upstream reference site in the Bow River (REF) were caged in 3 replicate raceways containing either 5% Pine Creek MWWE (PC) or Bow River water (BR; control) over 28 d. Liver transcriptomes were analyzed in male and female LND sampled on day 7, 14 and 28 from BR and PC, and compared to REF fish sampled on day 0. Concurrent with the caging, samples for selected ESOCs analysis were collected from BR and PC. Differently expressed unigenes (DEUs) in both BR and PC females vs males, increased over time and compared to REF LND fish. Moreover, DEUs in female and male LND within the same treatment (i.e., BR, PC) showed an increase over time compared to REF LND females and males. Time was the dominant factor affecting DEUs, whereas sex and treatment as variables had less of an impact on the hepatic transcriptome. Gene Set Enrichment Analysis of LND at BR vs PC over time revealed effects on genes involved in growth, metabolism of carbohydrates and lipids, and immune system on day 7; however, by day 28, 80-90% of the enriched genes were associated with tissue inflammation in both sexes. In this study, BR was used as the reference site, but the water system also consists of trace amounts of ESOCs originating from the two municipal wastewater plants discharging upstream of Pine Creek, which was confirmed with the ESOCs analysis. This highlights the challenges when identifying select gene bioindicators of effluent exposure. Additional efforts will confirm a subset of gene bioindicators of MWWE exposure that can be used in field-based ecotoxicogenomic studies in the Bow River and other water systems under anthropogenic stressors.

**2.15.P-Th154 Use of Riparian Spiders as Sentinels of Persistent and Bioavailable Chemical Contaminants in Aquatic Ecosystems**

***Matthew M. Chumchal**[1], Gale B. Beaubien[2], Ray Drenner[1], Madeline Pratt Hannappel[3], Marc Mills[2], Connor Olson[4], Ryan R. Otter[5], Andrew Charles Todd[1] and David Walters[6], (1) Texas Christian University, (2) U.S. Environmental Protection Agency, (3) University of North Texas, (4) Syracuse University, (5) Middle Tennessee State University, (6) U.S. Geological Survey*
Aquatic ecosystems around the world are contaminated with a wide range of anthropogenic chemicals, including metals and organic pollutants, that originate from point and nonpoint sources. Many of these chemical contaminants have complex environmental cycles, are persistent and bioavailable, can be incorporated into aquatic food webs, and pose a threat to the health of wildlife and humans. Identifying appropriate sentinels that reflect bioavailability is critical to assessing and managing aquatic ecosystems impacted by contaminants. The objective of the present study is to review research on riparian spiders as sentinels of persistent and bioavailable chemical contaminants in aquatic ecosystems. Our review of the literature on riparian spiders as sentinels suggests that significant progress has been made during the last two decades of research. We identified 55 published studies conducted around the world in which riparian spiders (primarily of the families Tetragnathidae, Araneidae, Lycosidae, and Pisauridae) were used as sentinels of chemical contamination of lotic, lentic, and estuarine systems. For several contaminants, such as polychlorinated biphenyls (PCBs), Hg, and Se, it is now clear that riparian spiders are appropriate sentinels. However, many contaminants and factors that could impact chemical concentrations in riparian spiders have not been well characterized. Further study of riparian spiders and their potential role as sentinels is critical because it would allow for development of national-scale programs that utilize riparian spiders as sentinels to monitor chemical contaminants in aquatic ecosystems. A riparian spider sentinel program in the United States would be complementary to existing national sentinel programs, including those for fish and immature dragonflies.

**2.15.P-Th155 Role of AhR and Oxidative Stress in the Regioselective Toxicities of Hydroxychrysenes in Embryonic Japanese Medaka**

***Philip Tanabe*** *and Daniel Schlenk, University of California, Riverside*

Oxygenated polycyclic aromatic hydrocarbons (oxy-PAHs) are environmental contaminants that can be created through oxidation of the parent PAH, many of which have been found to be more toxic than their parent compounds. Previous studies have found that 2-hydroxychrysene (2-OHCHR) and 6-hydroxychrysene (6-OHCHR) exhibit regioselective toxicities in embryonic Japanese medaka that was prevented by CYP inhibition, which reduced the formation of the 1,2-catechol, a potentially toxic metabolite prone to redox cycling and oxidative stress. 2-OHCHR has also been found to be a four-fold more potent aryl hydrocarbon receptor (AhR) agonist compared to 6-OHCHR. These findings led us to hypothesize that AhR activation and oxidative stress play an important role in 2-OHCHR toxicity. While treatments with the AhR agonists PCB126 and 2-methoxychrysene (2-MeOCHR) did not cause significant anemia or mortality, pretreatments with AhR antagonist CH-223191 reduced anemia by 97.2% ± 0.84 and mortality by 96.6% ± 0.69. AhR inhibition was confirmed by a significant reduction (91.0% ± 9.94) in EROD activity. Thiobarbituric acid reactive substances (TBARS) concentrations were 32.9% ± 3.56 higher ($p<0.05$) in 2-OHCHR treatments at 100 hpf compared to controls, indicating oxidative stress. Staining with 2',7'-Dichlorofluorescin diacetate (DCFDA) revealed 42.6% ± 2.69 of embryos exhibiting high concentrations of ROS in caudal tissues, which is a site for embryonic hematopoiesis. Both muscle and skeletal tissues were affected, as well as some caudal vasculature. These results indicate that AhR may mediate 2-OHCHR toxicity, upregulating CYP and potentially forming the 1,2-catechol that generates ROS in the embryos within caudal tissues, potentially disrupting hematopoiesis leading to anemia and subsequent mortality.

**2.15.P-Th156 Literature Review on the Use of Freshwater Bivalves in Bioaccumulation Field Studies.**

***Katie Reuff****, Lauren Koban and Amy Fowler, George Mason University*

This research explores current literature related to bioaccumulation field studies using freshwater bivalves, both native and invasive species, as indicators for chemical contaminants and highlight research gaps, ending with a call for future research directions. The literature analyzed relies on bioaccumulation, a type of biomonitoring that relies on morphological and behavior observation and biochemical alterations to assess environmental changes. For this study, only field studies of freshwater bivalves with bioaccumulation or trophic magnification factors were considered to reflect uptake from diet as well as environment. While bivalves have been used as bioindicators for pathogens and various pollutants in marine water, studies with bivalves in freshwater ecosystems are not only limited but often isolated to specific pockets of the world and an insufficient range of chemicals and species. As this literature review concludes, the ability of bivalves to bioaccumulate at lower concentrations indicates the powerful role they could play in assessing environmental threats.

**2.15.P-Th157 A Sediment Quality Triad Approach to Determine Benthic Condition of the Matagorda Bay System**

***Jasmine Caillier*** *and Paul Montagna, Texas A&M University-Corpus Christi*

The Matagorda Bay system is one out of seven estuaries along the Texas coast. Previously a diverse ecosystem, there has been a long-term decline in benthic abundance, biomass, and diversity. One possible explanation is contamination from municipal, agricultural, or industrial sources, which may be toxic to marine organisms. Degradation can be indicated by benthic integrity (i.e., diversity) or toxicity using the Sediment Quality Triad (SQT) approach, which includes contaminant concentrations as a measure of dose, a toxicity measure to assess biological effects, and benthic community diversity to indicate ecological integrity. Sediment samples from 24 sites were collected in the Matagorda Bay system to test the hypothesis that levels of chemical contaminants and benthic responses in sediments of Lavaca Bay will be higher than Matagorda Bay based on proximity to industrial inputs. The overall goal is to perform an SQT to determine which part of the estuary is being most affected, and assess bay health system wide.

**2.15.P-Th158 Various clinical signs of Japanese medaka (Oryzias latipes) in fish acute toxicity test and their link with moribundity and death**

*Takahiro Yamagishi, Haruna Watanabe and* ***Hiroshi Yamamoto****, National Institute for Environmental Studies, Japan*

Despite the revision of fish acute toxicity test (OECD guideline for testing of chemicals, No. 203) in 2019 to optionally observe clinical signs to encourage the use of moribundity as humane endpoints, not much information is available for the link between the observable clinical sublethal clinical signs and their transition to the imminent death, especially for Japanese medaka (*Oryzias latipes*), which is considered as one of major fish species used for the testing. Therefore, we established the criterion of the moribundity state based on the determination of death/clinical sign ratio for 13 signs observed in eight chemicals including typical reference chemicals, pesticides, pharmaceuticals, and metals, tested in this series of the study. Three of the observed clinical signs with death/clinical signs of 1; recumbency, lethargy, and floating at the surface; are considered as the prospects of the moribund state. We also found that the time from the first observation of each clinical sign to the transition to imminent death (survival time) was determined. By evaluating this survival time, the appropriateness of the timely euthanasia for the other 10 signs to reduce the time of suffering of the fish in the period before death. We also prepared a movie to define these clinical signs and made it available at our website.

**2.15.P-Th159 BMAA Affect on Adult Zebrafish Nervous System and Locomotion**

***Dani J. Hamilton*** *and Sherri A. Emer, Florida Gulf Coast University*

Beta-N-methylamino-L-alanine, or BMAA, is produced by cyanobacteria and can be considered neurotoxic at concentrations of 10-30 nM. Increases in cyanobacteria (blue green algae) blooms can result in increased BMAA in the environment. While previous experiments show neurotoxic effects of BMAA on developing fish embryos, there are limited data on the long term effects in adult fish. A FGCU pilot study of BMAA effects on adult zebrafish resulted in negative effects on nervous tissue and swimming behavior. Our goal was to find a BMAA concentration that enabled the analysis of central nervous system (CNS) anatomy and corresponding behavior. Adult zebrafish were exposed to zero, low, and high concentrations of BMAA, based on local concentrations reported for Sarasota Bay. Fish swimming behavior was recorded and analyzed throughout the 30-day exposures, and following exposures, we used immunohistochemistry and fluorescence microscopy to examine morphology, apoptosis, and degradation in the CNS with markers for the proteins caspase (cell death) and p-tau (degradation). Significant increases were observed in labeled caspase and p-tau in fish exposed to low and high BMAA concentrations compared to controls, and swimming speed and distance may decrease with exposure time. We are currently replicating the exposures to verify these findings and investigate the possibility of recovery. These experiments will help us understand the relationship of BMAA to neurodegeneration and behavior, while collecting toxin data that may be applicable to both wildlife and human health.

**2.15.P-Th160 Toxicity of Saturate, Aromatic, and Resin Fractions of Crude Oil to Palaemonetes pugio**

***Olivia Hernandez***[1]*, Emily C. Pisarski*[2] *and Ed Wirth*[2]*, (1) Oak Ridge Institute for Science Education (ORISE), (2) National Oceanic and Atmospheric Administration (NOAA)*

Determining the source of toxicity in crude oil is important when assessing risk to aquatic life. Upon release into the environment, various weathering processes alter the chemical and physical properties of crude oil. One weathering process that has been understudied, but recently found to play a larger role in oil spill weathering, is photo-oxidation. Previous work within NOAA’s National Centers for Coastal Ocean Science (NCCOS) has demonstrated that there is enhanced toxicity to larval estuarine organisms when exposed to ultraviolet (UV-A) light and thin oil sheens (<4µm), however, the compounds that are driving this enhanced toxicity are not well documented. This study explores the toxicity of the saturate, aromatic, and resin fractions in order to determine if toxicity shifts from fraction to fraction over time as oil is exposed to UV light. Louisiana Sweet Crude oil was exposed to either UV and no-UV conditions, then collected, fractionated and analyzed at three timepoints. A standard 96 h acute toxicity assay, with larval grass shrimp (*Palaemonetes pugio*) was performed using the crude oil fractions at each of the three timepoints. Whole oil sheen exposures were run concurrently alongside the fractions to validate our methodology. Nominal dosing concentrations of 15µm, 10µm, 4 µm, 1 µm and 0.25 µm sheens are reported and are reflective of previous NCCOS research in which LC50 toxicity values were obtained for a variety of estuarine species.  Gas chromatography mass spectrometry (GC/MS) analysis was used to quantify chemical constituents in each treatment for select toxicity tests (specifically polycyclic aromatic hydrocarbons, or PAHs). Results from this study will be used to assist understanding when responding to oil spills and how to better manage them in the future.

**2.15.P-Th161 An imminent problem of pollutants: Chemicals with epigenetic and transgenerational effects**

***Yi Yang**, HKU, China*

Recent mammalian studies have revealed that some endocrine disrupting chemicals (EDCs) can modify the epigenome by DNA methylation, histone modification or miRNAs interference, resulting in adverse transgenerational effects on subsequent generations (e.g. deformities, reproductive impairments and infertility), even though these offspring have never been exposed to EDCs throughout their whole life. *In vitro* studies carried out by our group showed that certain EDCs could modify the epigenome and potentially transmit the epigenetic changes through the human female and male germ lines. Arguably, chemicals that can cause epigenetic alterations and transgenerational reproductive impairment might pose a dramatic and long-lasting threat to the sustainability of the species.

Using the marine medaka (*Oryzias melastigma*) as a fish model, this project set out to test the hypothesis that F0 exposed to environmental realistic concentration of certain EDCs can cause epigenetic alterations, leading to transgenerational reproductive impairment in both males and females in the subsequent generations (F1 to F3). Four EDCs commonly found in elevated concentrations in coastal waters of PRD and China were selected, and their transgenerational effects in association with Darwinian fitness traits (including gametogenesis, sperm number and motility, ovarian atresia, reproductive hormones and expression of related genes along the HPG axis, fecundity, onset of puberty, fertilization success, sex ratio, apoptosis and cell proliferation) are being studied. In parallel, high-throughput massively parallel sequencing will be used to reveal the epigenetic mechanism (i.e., miRNA profile, global and gene specific DNA methylation patterns) underlying the observed reproductive impairments.

This research will enable us to coin a new class of pollutant with epigenetic and transgenerational effects, which is likely to attract global concern. The transgenerational effects revealed will compel a re-evaluation on the environmental and public health risks of these EDCs. Since endocrine and epigenetic regulations are highly conserved in vertebrates, our novel discoveries in this study will also shed light on epigenetic and transgenerational effects of epigenetic modifiers on higher vertebrates, including humans.

**2.15.P-Th162 A Systematic Review of the Joint Toxicity of Microplastics and Pesticides on Freshwater Organisms**

***Kyle Zachary Carver**, SRC Inc.*

As microplastics and environmental pollutants, such as pesticides, continue to pose potential dangers to freshwater ecosystems, it is important to understand how they might influence one another's toxicity. This systematic review examines how the toxicity of polyethylene/polystyrene microplastics is influenced by the presence or absence of organochlorine/organophosphate pesticides in freshwater fish/anurans, invertebrates, and algae. Following the Navigation Guide framework, literature searches were conducted in Scopus, Web of

Science, ProQuest, and Google Scholar. Of the 1365 articles found across the databases, eleven studies met the inclusion criteria and were assessed for strength of evidence and potential risk of bias. Nine of the eleven studies indicated strong evidence of the ecotoxicity of microplastics being modified by the presence of pesticides. While the primary outcome of interest (mortality) was not consistently affected, consistent and significant sub-lethal effects were seen across most of the body of evidence. All studies also demonstrated sound methodology and laboratory settings which controlled for any factors of concern. The topic of this review is still relatively understudied and should be further investigated and possibly expanded to examine this association in other species. Results of these studies collectively could have implications for a wide range of aquatic ecosystems and water quality overall.

**2.15.P-Th163 Optimizing a Synthetic Seawater Diluent for Microtox Toxicity Testing in Marine Systems**
***Rosemary Elizabeth Vichi**, Lance T. Yonkos and Dana K. Sackett, University of Maryland, College Park*
Microtox® is a bioassay testing system that utilizes *Aliivibrio fischeri*, a marine bioluminescent bacterium that produces light as a by-product of cellular respiration, to measure toxicity of environmental samples. Disruption of *A. fischeri* metabolic function corresponds to a reduction in light production measured by the Microtox system. Existing literature suggests Microtox has primarily been used in freshwater systems to measure toxicity in runoff, industrial discharge, and drinking water. Despite the importance of understanding toxicity in marine systems, the standard diluent provided for Microtox analysis, a 2% NaCl solution, may not be appropriate for testing toxicity in marine samples. Microtox assays using the standard diluent to reactivate *A. fischeri* prior to incubation in field-sourced marine samples (35 ppt salinity) resulted in substantial light gains, which is uncharacteristic for this test. This result is undesirable as a modest light gain may be interpreted as a non-toxic response, even if it truly reflects a toxicity-induced reduction in light gain compared to a higher baseline. We hypothesize that an increase in osmotic pressure upon introduction to the 35 ppt marine samples after activation in the 2% NaCl diluent is contributing to the increased metabolic activity, or light gain. We also hypothesize that the 2% NaCl diluent is deficient in numerous macro and micro nutrients compared to the marine samples; thus activation in the standard diluent results in suboptimal light production. To test our hypotheses we compared Microtox results using the 2% NaCl diluent to results using a 35 ppt synthetic diluent (standard diluent augmented with Crystal Sea® Marinemix bioassay formula). The augmented diluent outperformed the standard diluent, with *A. fischeri* producing more light upon activation and after the 15-min incubation interval. Likewise, retesting of field-sourced marine samples using the 35 ppt synthetic diluent showed substantial improvement in test performance, with significantly smaller light gains compared to activation with the 2% NaCl diluent. Remaining modest light gains when testing marine samples using the augmented diluent suggests the importance of additional micronutrients present in naturally-sourced marine media. Further optimization of the diluent to better reflect the typical constituents of seawater will be needed to ensure Microtox assays can accurately assess toxicity in marine samples.

**2.15.P-Th164 Do Dietary Sampling Method and Season Influence Estimated Selenium Concentrations in Boreal Freshwater Fish?**
***Maira Peixoto Mendes**, Beatriz Cupe Flores and Karsten Liber, University of Saskatchewan, Canada*
Selenium (Se) is an essential micronutrient with a narrow essentiality-toxicity window known to bioaccumulate in aquatic food webs. Selenium concentrations above essential levels have been associated with impaired reproduction, development and lethality, particularly in egg-laying vertebrates. Despite extensive research, the modelling of Se transfer and bioaccumulation potential in aquatic ecosystems still represents a great source of uncertainty for Se risk assessment. This study aimed to predict Se body burdens in three fish species (northern pike, white sucker and slimy sculpin) inhabiting boreal lakes (Vulture and McClean) downstream from the McClean Lake uranium milling operation in northern Saskatchewan. In addition, we investigated the potential effects of periphyton and macroinvertebrate sampling methods (artificial substrates vs sediment grab samples) and seasons (summer vs winter) on the predicted Se concentrations in freshwater fish (whole body and muscle). During the summer of 2019, periphyton and benthic macroinvertebrates (BMI) were sampled using artificial

substrates (n=4) and sediment grab samples (n=3) at 10 sampling stations (two in Vulture Lake and eight in McClean Lake). In winter 2021, periphyton and BMI were sampled through ice holes (n=3) using grab samples (n=4) in McClean Lake. Invertebrates were sorted to the lowest practical taxonomic level, and trophic transfer functions (TTF) were calculated for each taxon as the ratio of Se concentrations in BMI and dietary Se concentrations. Significantly higher Se body burdens were calculated using BMI collected from the artificial substrates compared to those calculated using BMI from sediment grab samples. Most importantly, predicted Se concentrations in northern pike and white sucker calculated based on BMI sampled with grab samples, but not artificial substrates, were comparable to measured Se concentrations in those species. The lack of available information on derived Se trophic transfer and conversion factors (whole body-muscle) for slimy sculpin, associated with sparsely measured Se concentrations in this species, increased the uncertainty around the predicted Se concentrations. No seasonal effect was identified in this study, but the limited number of winter sites sampled prevented a complete assessment of seasonal influences on Se bioaccumulation in BMI.

**2.15.P-Th165 Mummichog Fecundity and Size-Based Oocyte Enumeration as Indicators of Environmental Quality**

***Anna Kate Heider****, Alejandra (Alex) Vazquez and Lance T. Yonkos, University of Maryland, College Park*

The mummichog *Fundulus heteroclitus* is one of the most abundant intertidal fish species along the east coast of North America. Mummichog have a small home range (<300m), are hardy and easily field-collected, have large durable eggs, and thrive in urbanized and chemically polluted coastal areas, making them an ideal species for investigating local-to-regional environmental quality. Because they are short-lived (≤4yrs), mummichog are also useful for monitoring effectiveness of remediation efforts at improving environmental quality. Mummichog spawn sequentially throughout spring and summer with spawning synchronized to high water-associated spring tides (e.g., full and new moon). We hypothesize that enumeration of oocytes at various size categories prior to peak spawning events will allow estimation of total seasonal fecundity, and further, that differences in seasonal fecundity will provide insight into overall environmental quality. Approximately 3 days prior to full moon-associated spring tides during May, June, and July, 2021, ten mature female mummichog (≥65mm) were collected from 4 spatially-distinct locations (≥3km apart) within an historically contaminated tidal river. Fish were euthanized, weighed and measured, and ovaries excised and weighed (along with expressed eggs) for calculation of gonadosomatic index. Ovaries were placed in Gilson's Fluid (≥4wks) to weaken stromal tissues and facilitate disaggregation by washing through a series of sieves of mesh sizes: 1.4mm, 1.0mm, 0.7mm, and 0.3mm. Oocytes caught on progressively finer sieves were placed in separate petri dishes, imaged and enumerated. Resulting oocyte counts were used to investigate spatial and temporal trends in abundance within and across location, collection time, and oocyte size. Samples from all locations demonstrated maximum numbers of spawnable oocytes in May and June, followed by significantly decreasing numbers in July. However, the absolute abundance and relative change in abundance of spawnable oocytes between sample periods were markedly different between locations. Patterns of abundance of small and medium oocytes during May and June were not reflected in abundance of medium and large oocytes, respectively, in June and July collections. Additional research, with samples collected at more frequent intervals may be necessary to identify relationships between size-based oocyte abundances and future spawning output, and to identify optimal collection times for greatest benefit.

**2.15.P-Th166 Effect of Acute 6PPD-quinone Exposure on Cardiorespiratory Physiology of Salmonids of Differing Sensitivity**

***Summer Selinger*[1]***, Alper James Alcaraz[1], David James Montgomery[1], Xiaowen Ji[1], Steve Wiseman[2], Markus Hecker[1], Lynn Weber[1], David Janz[1] and Markus Brinkmann[1], (1) University of Saskatchewan, Canada, (2) University of Lethbridge, Canada*

N-(1,3-Dimethylbutyl)-N′-phenyl-p-phenylenediamine-quinone (6PPD-quinone) is a transformation product of the most widely used rubber tire antioxidant, 6PPD. Commonly found in road-way runoff, this compound has been reported to cause acute lethality at roughly ≤ 1 ug/L in a variety of salmonid species including coho salmon, rainbow trout, and brook trout. However, additional studies have shown other salmonid species such as arctic char and bull trout to be insensitive, even at significantly greater concentrations (20 ug/L). Sensitive species show distinctive symptoms including gasping, spiraling, increased ventilation, and loss of equilibrium, suggesting a possible impact on cardiorespiratory physiology. Here, we investigated the acute cardiorespiratory effects of 6PPD-quinone to two salmonids of differing sensitivity: a sensitive species, rainbow trout, and a tolerant species, Arctic char. Fish were exposed to 1 ug/L or 10 ug/L 6PPD-quinone in respirometry chambers for 48h to assess temporal changes in resting oxygen consumption compared to unexposed controls. Following exposure, cardiac ultrasound was used to characterize cardiac function by analyzing changes in ejection velocity, stroke volume, ventricular and atrial contractile rates, and cardiac output. Furthermore, electrocardiography was used to determine changes in the heart's electrical activity, and blood gas analyses was used to analyze changes in 19 parameters of the blood. Preliminary data show that 6PPD-quinone exposure does not significantly impact oxygen consumption rates in either species. In contrast, exposure appears to cause a significant increase in passive ventricular filling in both Arctic char and rainbow trout. In addition, in just rainbow trout, a decrease in end systolic volume and increase in atrial and ventricular contractile rates were observed, providing evidence of sympathetic stimulation. Cardiorespiratory symptoms observed in rainbow trout exposure might partly be driven by a significant increase in methemoglobin, resulting in an impaired ability to oxygenate the tissues. This is the first study to analyze the toxicity of 6PPD-quinone to the cardiorespiratory system of fishes of commercial, cultural, and ecological importance at environmentally relevant concentrations and provides information invaluable to a better understanding of the mechanism of 6PPD-quinone toxicity.

**2.15.P-Th167 A Transcriptomic View of 6PPD & 6PPD-Q in Pimephales promelas**
***Kendra Bush**, Monique Hazemi, John Hoang, Michelle Le, Brett R. Blackwell, Kevin Flynn, Emma Stacy and Dan Villenueve, U.S. Environmental Protection Agency*
Over the past few years, the world of aquatic toxicology has set its eyes on the chemical N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) and its transformation product N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-quinone), both of which are present in urban runoff and impact coho salmon (*Oncorhynchus kisutch*) populations. While these chemicals have been recognized as environmental contaminants of concern, a conclusive mode of action for their toxicity has yet to be elucidated. This study utilized a cost- and time-efficient assay to explore the apical and transcriptomic impacts of both 6PPD and 6PPD-quinone on the standard freshwater test species, fathead minnow (*Pimephales promelas*). Five-day post-hatch larval fish were exposed to eight concentrations (½ log spacing) per chemical in 1 mL, 96-well plates for 24 hours at 25°C under a 16:8 hour, light: dark photoperiod. Post-exposure survival was enumerated to determine LC50 values. The LC50 values for 6PPD and 6PPD-quinone calculated from this study were $8.60 \times 10^4$ µg/L and $>2.64 \times 10^5$ µg/L (test high) respectively compared to the published 250 µg/L and 0.79 µg/L for coho salmon. This is consistent with other species studied that have shown higher 6PPD-quinone LC50 values of >14.2 µg/L in arctic char (*Salvelinus alpinus*) and white sturgeon (*Acipenser transmontanus*) and 308.67 µg/L for zebrafish (*Danio rerio*). Total RNA was extracted from individual fathead minnow and a transcriptomic library was produced and sequenced. The resulting transcript abundance data were analyzed using BMDExpress3 and a custom analysis pipeline in R statistical software to obtain transcriptomic points of departure (tPODs) and a list of differentially expressed genes (DEGs). Both the apical and transcriptomic results suggest that fathead minnows are more sensitive to 6PPD than 6PPD-quinone. However, no mode of action was suggested by the list of DEGs. It is unclear whether the results of this high throughput assay suggest a non-specific mode of action or simply a lack of sensitivity of larval fathead minnows to 6PPD and 6PPD-quinone. *The contents of this abstract neither constitute, nor necessarily reflect, official US EPA policy.*

**2.15.P-Th168 Abnormalities of an At-Risk Amphibian: Could Cyanobacteria be Involved?**
*Kiersten Nelson[1], Raven Bier[1], Heather Latham[1], Adam McFall[1], Edward Tucker Stonecypher[1], Christian Swartzbaugh[1], Scott Weir[2] and **Stacey L. Lance**[1], (1) University of Georgia, (2) DAWSON*

Amphibians are an important part of wetland ecosystems and are often studied to examine the impacts of contaminants on aquatic environments. At the same time, they are experiencing global declines and captive rearing is being used to manage many species. One such species, the Carolina gopher frog (*Lithobates capito*), is being head-started across multiple facilities in three U.S.A. states for the purpose of population augmentation and/or reintroduction. In 2021, we reared gopher frog tadpoles in mesocosms to augment populations in South Carolina, U.S.A. Survival was high (95%) but almost all (99%) juveniles emerged with at least one of a suite of developmental abnormalities affecting skin, eyes, jaws, and gills. Two other head-starting facilities also reared gopher frogs in 2021 that exhibited the same abnormalities, but in lower frequencies. In an initial attempt to determine potential causes of these abnormalities we collected water samples from mesocosms across seven rearing facilities to compare water chemistry in mesocosms with and without abnormal frogs. Additionally, we ruled out common causes of abnormalities, such as pathogens. While water chemistry varied greatly across facilities, there was no relationship to presence or severity of abnormalities. Previous studies have suggested that retinoids, secreted by cyanobacteria, may be an important, but largely ignored, cause of amphibian abnormalities. To address this possibility, we set up a factorial microcosm experiment examining the plant material used as substrate in gopher frog mesocosms. We used seven sources of substrate plus a no substrate control, three time points, and four replicates for a total of 96 microcosms. We destructively sampled microcosms at 2, 7, and 10 weeks and filtered water for nutrient analysis and systematically sampled periphyton from the substrate. Phosphorus levels in leachate collected at two weeks ranged from 24 to 1172 ppb, with higher levels in microcosms containing substrate associated with gopher frog abnormalities. The low N:P ratios in these microcosms would create an environment conducive to proliferation of nitrogen fixing cyanobacteria. We are currently using quantitative PCR to estimate the relative abundance of cyanobacteria and are sequencing to characterize the microbial communities. We discuss the abnormalities observed, the potential role of cyanobacteria, and the implications for wetland ecosystems in general and amphibian conservation specifically.

**2.15.P-Th169 Temperature and UV Interactions: Implication for Phototoxicity in Fish**

***Alexis Khursigara**, Rachel R. Leads and Aaron Roberts, University of North Texas*

In the natural environment, fish are rarely exposed to one stressor at a time. Previous research has shown that polycyclic aromatic hydrocarbons (PAHs) are phototoxic, a phenomenon in which PAHs become more toxic in the presence of ultraviolet (UV) radiation. However, little is known about the effects of additional stressors on phototoxicity. To date, only one study has looked at the effects of temperature on phototoxicity in early life stage fish. The goal of this study is to understand the interactive effects of temperature and UV radiation, and the implications it can have for phototoxicity in early life stage fish. In an initial study, larval zebrafish were exposed to artificial UV light or fluorescent lighting at three different rearing temperatures for 96 hours following fertilization. UV exposure and temperature had a significant interactive effect on the number of fish hatched after 96 hours. Total length, standard length, spine length, eye area, and iris area in surviving fish were affected by either temperature or UV. Only yolk sac area was significantly affected by temperature and UV. Given that previous work has shown that UV and oil co-exposure impair eye development, and eye development was impacted by temperature, the additional stress of varying temperatures are likely to exacerbate these effects of phototoxicity on eye development.

**2.15.P-Th170 Impacts of Microplastics on the Growth and Activity of the Ammonia-Oxidizing Bacterium Nitrosospira sp. Strain AV**

***Mara Faith Walters** and Bongkeun Song, Virginia Institute of Marine Science, William & Mary*

Microplastics are ubiquitous in the environment and their toxicity has been reported in a diverse array of organisms. Among these organisms are the microbes responsible for the processes that govern the nitrogen (N) cycle. Within the N cycle processes, nitrification is a key pathway converting ammonium to nitrite and then nitrate which is essential for nutrient bioavailability and denitrification in various environments. A previous study has shown that microplastics can affect the nitrification activities of sedimentary microbial communities. However, it is unclear which specific nitrifying microbes are being negatively impacted by exposure to microplastics of different polymer types. Additionally, it is unclear whether UV-weathered microplastics will affect nitrifying microbes differently than non-UV-weathered ("pristine") microplastics. To begin to answer these outstanding questions, we conducted an exposure experiment with cultures of the ammonia-oxidizing bacterium *Nitrosospira sp.* strain AV over the course of five days. Pristine or UV-weathered microplastics composed of polyethylene (PE), polylactic acid (PLA), polyurethane foam (PUF), or polyvinyl chloride (PVC) were added to cultures at a concentration of 1 mg/mL. Changes in bacterial cell numbers and concentrations of nitrite and nitrate were measured over the course of the exposure to determine impacts on bacterial growth and nitrification rates, respectively. Bacterial RNA was extracted and used to quantify the expression of the 16S rRNA gene and the ammonia monooxygenase (*amoA*) gene that encodes for ammonia oxidation to nitrite as another measurement of cellular growth and activity. Our results suggest that the impacts of microplastics on nitrification vary by polymer type and with UV-weathering. Overall, this study confirms that microplastics can affect microbial nitrogen cycling and indicates the plastic polymer types of greatest concern for nitrifying microbes. Future studies will investigate the impacts of microplastics on additional ecologically important species of ammonia-oxidizing bacteria and archaea, as well as nitrite-oxidizing bacteria, to better understand microplastic impacts on nitrification and related processes.

**2.15.P-Th171 Bioavailability of Heavy Metals in Waters Impacted by Mining Waste and the Tar Creek Superfund Site**

***Stacey Herriage***, *Matteo Minghetti and Jason B. Belden, Oklahoma State University*

The Tri-State Mining District (TSMD), composed of the region surrounding northeast Oklahoma, southwest Missouri, and southeast Kansas, has been the major source of heavy metals entering the Grand Lake watershed since the mid-19$^{th}$ century, with the largest contribution of contaminants being from the area now known as the Tar Creek Superfund site. This study aims to evaluate the impact of heavy metals on fish populations in waterways impacted by mining waste surrounding the superfund site, with a specific emphasis on the development of a novel biosensor based on the fish gill cell line derived from rainbow trout (*Oncorhynchus mykiss*), the RTgill-W1. As a first step, we wanted to evaluate heavy metal bioavailability in these waters. So far, water and sediment samples have been collected from 7 sites that represent the radius of flow from bodies of water closest to the source of contamination to the southward direction, which we predict will reflect a gradient of change in the concentrations of heavy metals as they reduce. Water and sediment samples are being first analyzed for heavy metals. Additional water chemistry parameters will be used to predict bioavailability using the Biotic Ligand Model (BLM). An *in vitro* method for measuring cytotoxicity has been performed by exposing RTgill-W1 cells to the sample water. The cytotoxicity assay results have been as expected. A significantly reduced viability by as much as 80% was observed in cells exposed to the water collected from the sources closest to mining sites whereas cytotoxicity in reference sites, was identical to that of controls (i.e., cell exposed to EPA moderately hard water). The preliminary data show that metal concentrations in water sampled from some sites exceed EPA water quality criteria. For example, concentrations of dissolved Zn greater than 4000 µg/L were measured in samples from Tar Creek. Although the BLM data is still in progress, the cytotoxicity data confirms that the metal concentrations in those waters are elevated sufficiently enough to produce toxic effects. Interestingly, we have found a variety of fish species and other aquatic organisms in the most contaminated waters which suggest that these organisms must have evolved specific mechanisms to tolerate metal toxicity.

**2.15.P-Th172 Identifying Fish Photolocomotor Behavioral Response Profiles and Gene Expression Changes for the Cyanobacterial Toxin, Cylindrospermopsin**

***Kendall Rose Scarlett***[1], *Lea Marie Lovin*[1], *Laura M. Langan*[1], *Sujin Kim*[1], *Kevin Stroski*[1], *Saurabh Chatterjee*[2], *Thad Scott*[1] *and Bryan W. Brooks*[1], *(1) Baylor University, (3) University of South Carolina*

Although diverse cyanotoxins are produced during harmful algal blooms (HABs), their direct impact on behavior such as swimming is less studied. Therefore, we examined the influence of cylindrospermopsin (CYN), a cyanotoxin that appears to be increasingly prevalent in aquatic systems around the globe, on swimming activity of the zebrafish and fathead minnow models during interchanging photoperiods, and further examined changes in gene expression using RT-qPCR. Common standardized guidelines for toxicity studies with zebrafish (OECD) and fathead minnows (US EPA) were used to facilitate the comparability of our findings to other studies. Behavioral observations occurred for 50 minutes, with 10 minutes of acclimation, two 10-minute dark periods, and two 10-minute light periods. Selection of CYN treatment levels were informed by our previous probabilistic assessments of global occurrence in surface waters. Cycloheximide, a model protein synthesis inhibitor, was used as a positive control, and treatment levels were determined from initial rangefinder and behavioral assays prior to experimentation. Total zebrafish locomotor activity was significantly reduced ($p<0.10$) at the highest concentration (1660 μg/L) during the dark cycles. Overall significant changes for fathead minnows were observed at the highest two concentrations (830 and 1660 μg/L) during the light cycles. Zebrafish photomotor behavior was significantly altered during the first dark phase ($p<0.05$) at multiple treatment levels, while fathead minnows were significantly affected at the highest concentration during the first dark cycle ($p<0.01$). Further analyses in these common fish models focused on molecular consequences of CYN on various genes involved in oxidative stress (e.g., gpx1a, sod1), hepatotoxicity (e.g., fabp10a, cyp3a65) neurotoxicity (e.g., neurog1, tuba1a), and DNA damage (e.g., bax, gadd45b). Considering that toxins and other contaminants co-occur across pronounced salinity gradients, future work is warranted at different salinities to inform assessment and management of cyanobacterial toxins across the freshwater to marine continuum.

**2.15.P-Th173 The Effects of Nanomaterials on the Metabolism and Nutrient Excretion in the Freshwater Snail, Physella acuta**

***Charles Mansfield***[1]*, Brittany Grace Perrotta*[2]*, Ryan King*[1] *and Cole W. Matson*[1]*, (1) Baylor University, (2) McMaster University, Canada*

*Physella acuta* are freshwater snails that play an important role in the ecological stoichiometry of their microenvironments. Via their grazing behaviors, these snails consume and digest periphyton and release bioavailable forms of nutrients back into their microenvironment in a process called consumer mediated nutrient recycling. Previous research has indicated a correlation between the presence of grazers, in this case freshwater snails, and an increase in total ecosystem biomass. However, this nutrient recycling was shown to be dependent upon the availability of nutrients in the surrounding environment. In a study performed by Perrotta et. al (2020), snails occupying a nutrient limited environment showed reduced levels of nutrients (nitrogen and phosphorous) in their excretions, which will likely reduce the overall nutrient levels in the microenvironment. Snails occupying a high nutrient environment showed little to no decrease in the nutrients they excreted into their environment. The same freshwater microcosms exposed to differing nutrient regimes (high and low) ecosystems were co-exposed to anthropogenic contaminants (copper and gold nanoparticles), the excretion behavior of the low-nutrient snails was significantly increased. *P. acuta*, when exposed to nanoparticles, ceased regulating the levels of nutrients they excreted into the environment. These data suggest a previously unknown mechanism of anthropogenic eutrophication, indirectly caused by perturbations in the snails' excretory pathways. It is well established that nanomaterials are released in wastewater effluent and industrial runoff. Furthermore, wastewater effluent is known to be high in nutrients, increasing the likelihood of these snails being exposed to high nutrient environments, in the presence of both periphyton and nanomaterials. To the best of our knowledge, there is little data investigating the underlying mechanism of nutrient excretion or impacts of nanomaterial exposure on the feeding behavior and metabolism of gastropods. Using RNA-sequencing, this research seeks to identify the genes associated with nutrient recycling and feeding behavior in the freshwater snail *Physella actua* to identify the molecular pathway in which nanomaterials interact with to alter nutrient excretion.

**2.15.P-Th174 Plastic Debris in Sai Gon - Dong Nai Rivers and Potential Impacts on Aquatic Organisms**

***Bao-Son Trinh***[1]*, Tham C. Hoang*[2] *and Gunther Rosen*[3]*, (1)Vietnam National University of Ho Chi Minh City, Viet Nam, (2) Auburn University, (3) Naval Information Warfare Center (NIWC) Pacific*

Plastic debris is one of the most pressing environmental issues in Viet Nam and over the globe. This study investigated, on one hand, concentrations and types of plastic debris, including macroplastics (MaPs, > 5.0 mm) and microplastics (MiPs, 0.3–5.0 mm), in surface water and sediment of Sai Gon, Dong Nai rivers and Can Gio estuary in the South of Viet Nam. Samples were collected at 8 sampling sites located from upstream to downstream during monsoon of 2021-2022. Concentrations of MaPs and MiPs in surface water were determined to be low at the upstream sites (S1 and S4), significantly increased at the downstream sites (S2, S3 and S5), and decreased at the estuary sites (S6, S7 and S8). The major types of MaPs and MiPs in surface water determined by FTIR-ATR technique were PE, PP, and PS while other types, e.g. PET, PVC, PU, nylon, silicon, etc., were also identified. Nevertheless, concentrations of MaPs and MiPs in sediment were not significantly low at the upstream sites (S1 and S4), increased at the downstream and estuay sites (S2, S3, S5, S6, S7, and S8). The major types of MaPs and MiPs in sediment were PE, PP, and PET while various other types were also identified. On the other hand, potential impacts of MiPs on typical aquatic organisms were also conducted in laboratory. River giant prawn, *Macrobrachium rosenbergii*, and white hard clam, *Meretrix lyrate*, were separately exposed with different concentrations of PE fluorescent microbeads (63–75 µm diameter) for up to 28 days to observe the accumulation of microbeads in these species. It is reported that the higher PE microbead concentrations were exposed, the higher PE microbeads were accumulated in both prawn and clam. The correlations of the exposure time and PE accumulation were linear regression for the assays with prawn ($y = 7.426x$, $R^2 = 0.8672$ in the treatments with high PE concentration) but non-linear regression for the assays with clam ($y = - 23.828x^2 + 138.4x$, $R^2 = 0.2681$ in the treatments with high PE concentration). In general, while plastic debris, particularly MiPs, were significantly identified in surface waters and sediments of Sai Gon, Dong Nai rivers and Can Gio estuary, MiPs can be accumulated in typical aquatic organisms living in this areas.

## 2.15.V Late Breaking Science: Aquatic Toxicology, Ecology and Stress Response

### 2.15.V-01 Microbiome Analysis of Freshwater Fingernail Clams (Sphaeriidae) Exposed to Trace Metal Pollution

*Dechen Edwards[1,2], Jenifer B. Walke[1], Carmen A. Nezat[1] and **Joanna Joyner-Matos**[1], (1) Eastern Washington University, (2) University of Alberta, Canada*

Aquatic habitats impacted by anthropogenic activities such as mining can contain mixtures of trace metals; the consequences of chronic exposure to these mixtures on the host-associated microbial communities of benthic macroinvertebrates is poorly understood. Freshwater fingernail clams (*Sphaeriidae*) inhabit lakes that lie in the floodplain of the Coeur d'Alene River, which drains the "Silver Valley" region of northern Idaho (USA) that includes the Bunker Hill Mining and Metallurgical Complex Superfund site. We used whole-clam tissue cadmium burden to confirm that the clams were exposed to conditions that corresponded with historical patterns of sediment metal toxicity. We then compared the surface water microbial communities with the whole-body microbiome from clams collected from four metal-impacted lakes and from a reference lake to test the hypothesis that chronic exposure to a trace metal mixture influences selection of microbial community members. The five lake water microbial communities were indistinguishable and contained phyla that are typically found in freshwater systems, including Bacteriodetes, Firmicutes, Proteobacteria. In contrast, the paired clam tissue and surface water microbial communities were significantly different from each other, and clam microbial communities differed significantly across lakes. Results from this preliminary study are consistent with evidence of selection for microbial partners and suggest another potential strategy for tolerance of chronic exposure to metal mixtures.

### 2.15.V-02 Embryotoxic effects of pure and co-contaminated microplastics to early life stages of Danio rerio

***Gabriela V. Aguirre- Martinez**[1], Leandro Altamirano[1] and Lisette Zenteno-Devaud[2], (1) Universidad Arturo Prat, Iquique, Chile, (2) Universidad Católica de Santísima Concepción, Chile*

Microplastics (MPs) are considered as a global threat as they have been found in diverse aquatic environments. However little is known regarding the potential of MPs to carry organic hydrophobic contaminants and its effects on aquatic biota. The aim of this study was to investigate embryotoxic effect on *Danio rerio* after exposure to pure polyethylene (PE) microplastics (MPs), MPs contaminated with phenanthrene (MPs+PHE), and chlorpyrifos (MPs+CPF). Zebrafish (ZF) embryos were exposed, from 2 until 96-h post-fertilization to different sizes of MPs (1-5, 27-32, 45-53 µm) and to realistic concentrations (150, 1500 particles/mL). Lethal and subletal endpoints (malformation) were evaluated every 24h. Results indicate that embryos did not survive after 72h when exposed to MP+CPF (1-5 µm) at both concentrations tested. Mortality was observed at 96h when exposed to sizes 27-32 and 45-53 µm at 1500 particles/mL being the number of ZF significantly lower compared to control ($p<0.05$). Exposure to 1-5 µm MP+PHE induce mortality at 96h being significantly different to control ($p<0.05$). Although increased mortality was observed in embryos exposed to MP+PHE statistical analysis did not show significant differences between the control and sizes 27-32 and 45-53 µm at the same time of exposure. Significant decrease in hatching rate was noticed in embryos exposed to MP+CPF compared to control ($p<0.05$) at higher concentration tested, this response was similar for all sizes of MPs. Only exposure to MP+PHE (1-5 µm) at higher concentration induced significant decrease at hatching rate compared to control ($p<0.05$). Morphology alteration was observed after exposure to MP+CLP and MP+PHE however, statistical evaluation did not reveal significant differences between the control and experimental groups. Results of this study demonstrated bioavailability of CLP and PHE and release of these contaminants upon contact with organisms during experiments, being MPs+CPF more toxic than MPs+Phe. Overall, the findings of this study indicated that PE MPs induced embryotoxic effect on *Danio rerio* and this effect would depend not only on the concentration, exposure time, and size of the MPs but also on the contaminant carried by PE MPs. **Funding:** FONDECYT N°**11180466**, Nº3190455

### 2.15.V-03 Carbon Decomposition by Microbial Communities in a Constructed Wetland Treating Cu and Zn

***McKenzie Shea Cromer****, Corinne Sweeney and Raven L. Bier, Savannah River Ecology Laboratory, University of Georgia*

Microbial communities have an outstanding impact on nutrient cycling and environmental disturbances in terrestrial and aquatic ecosystems. Environmental toxins and metals can be influenced by the presence of microbial metabolisms throughout the seasons. It remains unclear how influential the diversity and rates of these metabolisms can be on controlling the release of metals into the environment. The current study compares the functional capability of microorganisms to change the environmental conditions that remove metals in the discharge effluent from a constructed treatment wetland. Bulrush (*Schoenoplectus californicus)* and wetland floc were collected between the inflow and outflow of two wetland cells from January through September. Samples were analyzed for metals retention of Cu and Zn. Carbon metabolisms rates and diversity were measured and indicated that the rate of microbial decomposition on bulrush decreased from the inflow to outflow. Monitoring the effectiveness of microbial decomposition in treatment wetlands can help us further minimize the ecological effects of industrial activities.

## Track 3: Wildlife Toxicology, Ecology and Stress Response

### 3.01.P Bridging ecotoxicology and risk assessment for aquatic and terrestrial plants

### 3.01.P-We083 Impacts of Amendments to Promote Mine Revegetation: Response of Perennial Native Plants

*Michael Bollman[1], **David Olszyk**[1], Marjorie Storm[2], Viola Manning[3], Kristin Trippe[3], Jeffrey Novak[3], Donald Watts[3] and Mark G. Johnson[1], (1) U.S. Environmental Protection Agency, (2 )CSS Inc., (3) U.S. Department of Agriculture*

Past mining activities in the United States have left a legacy of heavy metal contaminated soils that require remediation to allow for increased phytostabilization and reduced soil and water pollution. Of special concern to EPA's Region 10 is the Formosa mine superfund site located in south-central Oregon.  This site has a large area of acidic mine tailings where it is difficult to establish vegetation. In an *in situ* field study, we evaluated the effects of tilling the tailings (1) without adding any amendments, (2) with amendments (lime, biosolids) + potassium, (3) with amendments + biochar; and (1), (2) or (3) plus inoculation with locally-sourced microbes (LSM) on survival and growth responses of plants. Six perennial native plant species were used: *Anaphalis maragitacea*, *Carex inops*, *Chamerion angustifolium*, *Elymus glaucus*, *Lupinus sericeus*, and *Potentilla gracilis* with responses determined over two years. Enhanced survival and growth was positively related for all species in amended plots with an increase in tailing pH and decreased tailing concentrations of extractable metals (e.g., Al, Cu, Fe, Mn, Zn) compared with unamended plots. There were no LSM effects to date. *Elymus glaucus* grew especially well and became dominant in the amendments + biochar plots over time. This study highlights the potential for soil amendments developed under the U.S. Environmental Protection Agency, Office of Research and Development and U.S. Department of Agriculture, Agricultural Research Service biochar research programs to improve soil chemistry and enhance plant growth; thus increasing the potential for success of vegetation reestablishment on degraded soils and mining impacted soils in Superfund sites across the United States.

**3.01.P-We084 Examining the Use of Wetland Plants for Aquaculture Wastewater Effluent Remediation**
***Nicholas Blandford**[1], Leah Dickenson[1], Krista Robertson[1], Richard Grosshans[2], Bruce Hardy[3], Nazim Cicek[1] and Vince Palace[4], (1) University of Manitoba, Canada, (2) International Institute for Sustainable Development, Canada, (3) Myera Group, Canada, (4) IISD Experimental Lakes Area (IISD-ELA), Canada*
Aquaculture is among the fastest growing food production sectors in the world. However, aquaculture systems generate wastewater high in nitrogen and phosphorous. These effluents present a risk of eutrophication in receiving waters and threaten the health of aquatic ecosystems. Thus, a number of conventional wastewater treatment methods have been applied to aquaculture systems. Constructed wetlands are a promising and cost-effective strategy for treating wastewaters and offer the additional benefit of recycling nutrients that would otherwise be discharged to the environment. Here we provide details of recent studies showing that cattail (*Thypha* spp.) and northern wild rice (*Zizania palustris* L.), abundant wetland plants, can be implemented in the remediation of aquaculture wastewater effluent. Model wetlands were established in mesocosms (2 m diameter; ~1500 L) and had a gradient of aquaculture wastewater routinely applied over several weeks. An initial pilot-scale study was conducted using cattail wetlands which were found to be robust to changes in water quality and demonstrated the ability to buffer the impacts of nutrient loading. Minor increases (~0.15 mg $L^{-1}$) in each of total phosphorus, total dissolved phosphorus, and soluble reactive phosphorus were observed. As wetlands proliferated over time, phosphorous concentrations returned to background levels (< 0.05 mg $L^{-1}$). In a subsequent study, both wild rice and a combination of cattail and wild rice wetlands were investigated. The implementation of this remediation strategy demonstrates how nutrients can be recycled in wetland plants, and outlines the potential for aquaculture operations to be coupled to wild rice crop production in the future.

**3.01.P-We085 Non-Target Terrestrial Plant Testing and Risk Assessments for Pesticides**
***Clifford Habig**, Compliance Services International (CSI)*
The USEPA requires two non-target terrestrial plant (NTP) tests to support registration of outdoor uses of pesticides. These tests are the seedling emergence (SE) and vegetative vigor (VV) tests. The results of these tests are used to evaluate potential direct risks to non-target terrestrial plants as well as indirect risks to other terrestrial species, including potential impacts on species' habitat.

Both NTP tests include testing on at least four monocot species and six dicot species, and involve a single pesticide application. These are short-term tests, with in-life phases of 14 to 21 days. In the SE study, seeds are planted into a low percentage organic matter soil, followed by pesticide application to the soil. In the VV test, young, recently sprouted plants are directly treated with a foliar spray. Key endpoints in these studies include plant emergence, plant survival, and plant growth, measured as length and dry weight. Endpoints include EC25 values and NOECs for each species, along with observations of phytotoxicity; there are no endpoints, such as seed production, that directly evaluate potential effects on reproduction.

Current EPA NTP risk assessment procedures are based on the TerrPlant model, which includes default estimates of exposure through runoff and spray drift, with NTP risks being based on results for the most sensitive monocot and dicot species. Higher-tier risk assessments can be conducted using other EPA models, such as AgDrift and PRZM-EXAMS, to refine potential exposure. Examples of NTP risk assessments using these models will be presented and discussed. Additionally, possible options for evaluating potential effects on reproduction through seed production using fast-growing species such as radish and arugula will be discussed.

**3.01.P-We086 INHABIT for Broad-Scale Assessment of Invasive Terrestrial Vegetation Species**
***Jillian LaRoe****, Applied Analysis Solutions LLC*
The invasive species habitat tool, INHABIT, is a publicly available web tool developed by the U.S. Geological Survey (Fort Collins Science Center, CO) to model habitat suitability for terrestrial invasive vegetation species across the continental United States. Due to increasing challenges of managing invasive species as well as their ecological and economic impacts, best practices have called for proactive management to prevent spread. The primary challenge land managers are often presented with is that broad-scale assessments require comprehensive landscape surveys of species locations, and often little is known about the distribution of new invaders. INHABIT aims to fill the knowledge gap between known distributions of more than 140 invasive species and the potential risk they pose by modeling broad-scale habitat suitability (90-meter spatial resolution) at four different thresholds, from more conservative estimates to more prolific potential ranges. Using species distribution modeling techniques within a semiautomated workflow, the INHABIT maps ensemble five different modeling algorithms and two different background point selection methods using ecologically relevant predictors specific to each target species. The web tool provides users with the ability to investigate species relevant to different management boundaries, including distance metrics to known species occurrences and summaries of the suitable habitat within a given management area. The habitat suitability maps for each species are also publicly available for download to be used in other analyses. INHABIT development efforts were aimed to support the management objectives of the National Park Service; however, the results have the potential to support broad-scale proactive management of many invasive vegetation species (https://gis.usgs.gov/inhabit/).

## 3.02 Field Studies for Reducing Uncertainty in Contaminant Exposure and Effects Assessments for Wildlife

**3.02.T-01 Combining field and laboratory approaches to improve relevance of effects assessments for wildlife**
***Margaret L. Eng***[1]*, Christy Morrissey*[2] *and John E. Elliott*[1]*, (1) Environment and Climate Change Canada, Canada, (2) University of Saskatchewan, Canada*
Avian toxicity studies for risk assessments are typically conducted in laboratory settings using domesticated species. While these studies have the advantage of controlled exposures that can establish causality, the outcomes may not be representative for free-ranging wildlife species facing multiple stressors. Conversely, field studies that assess contaminant effects in relevant wildlife species are typically correlative, with exposures that may not be of toxicological interest, and the long-range movement and dispersal of individuals can limit the ability to monitor contaminant effects. To corroborate outcomes of laboratory studies, subsequent field studies could be conducted, and vice versa. Alternatively, integrating field and laboratory approaches into a single study has the potential to more efficiently assess contaminant effects in wildlife. Ideally, to increase relevance and reliability of effects assessments for wildlife, studies would be in a species of interest or a suitable surrogate, be in natural environments, have a range of toxicologically relevant exposure concentrations, and have the ability to monitor effects that can be linked to population level outcomes. This presentation will

discuss integrative approaches for improving the relevance of effects assessments for wildlife, and will highlight case studies where a combination of field and laboratory approaches were used to make causal linkages between contaminant exposure and effects in avian wildlife. Examples include the use of egg injections in wildlife species (European starling and gray catbirds) to assess effects thresholds for embryotoxicity of dioxin like compounds, and using controlled dosing and automated telemetry in migratory songbirds (white-crowned sparrows) to assess the effects of insecticide exposure on condition and behavior.

**3.02.T-02 Frontiers in Quantifying Wildlife Behavioural Responses to Chemical Pollution**

***Michael Bertram*** *and Tomas Brodin, Swedish University of Agricultural Sciences (SLU), Sweden*

Animal behaviour is remarkably sensitive to disruption by chemical pollution, with widespread implications for ecological and evolutionary processes in contaminated wildlife populations. However, conventional approaches applied to study the impacts of chemical pollutants on wildlife behaviour seldom address the complexity of natural environments in which contamination occurs. This talk will present a new review by Bertram et al. (2022, Biological Reviews, https://doi.org/10.1111/brv.12844), which aims to guide the rapidly developing field of behavioural ecotoxicology towards increased environmental realism, ecological complexity, and mechanistic understanding. This review identifies research areas in ecology that to date have been largely overlooked within behavioural ecotoxicology but which promise to yield valuable insights, including within- and among-individual variation, social networks and collective behaviour, and multi-stressor interactions. Further, the review features methodological and technological innovations that enable the collection of data on pollutant-induced behavioural changes at an unprecedented resolution and scale in the laboratory and the field. In an era of rapid environmental change, there is an urgent need to advance our understanding of the real-world impacts of chemical pollution on wildlife behaviour. This review therefore provides a roadmap of the major outstanding questions in behavioural ecotoxicology and highlights the need for increased cross-talk with other disciplines in order to find the answers.

**3.02.T-04 Effects Of Developmental Exposure To Chlorpyrifos On Hippocampal Cell Proliferation In A Migratory Bird**

***Sereena Moore*[1]*, Margaret L. Eng*[2]*, Christy Morrissey*[3] *and Mélanie Guigueno*[1]*, (1) McGill University, Canada, (2) Environment and Climate Change Canada, Canada, (3) University of Saskatchewan, Canada*

Migratory birds rely on the hippocampus, a brain region important for spatial cognition. Within the hippocampus, an increased production of new neurons may be linked to the higher demand on spatial cognition in migrants, as previous studies have shown that migratory birds have higher levels of neurogenesis and neuronal recruitment relative to non-migratory birds. However, neurotoxic insecticides, such as the organophosphate chlorpyrifos, can directly affect the brain and migration. For example, exposure to chlorpyrifos has been shown to reduce neurogenesis, DNA synthesis and number of brain cells in rodents, reduce neurogenesis in chicks, and additionally impair migratory orientation in a migratory bird. Yet, it is unknown how chlorpyrifos affects the development of the hippocampus in migratory birds. We examined how an acute exposure to chlorpyrifos affected hippocampal cell proliferation in a migratory population of European starlings (*Sturnus vulgaris)*. On post-hatch day (PHD) 1-3, we dosed nestlings with a single dose of either chlorpyrifos (6 mg/kg-bw) or a vehicle control and we measured body mass. The next day (PHD 2-4), we measured post-dosing body mass and we blood sampled all nestlings to measure acetylcholinesterase (AChE) inhibition, a biomarker of organophosphate exposure. Subsequently, we collected brains at three timepoints during the nestling period: early (PHD 5-7), middle (PHD 11-13) and late (PHD 19-20). We stained brain sections to label 5-ethynyl-2'-deoxyuridine (EdU) cells, an exogenous marker of cell proliferation, and counted EdU-positive cells within the hippocampus using unbiased stereology to determine the total number of hippocampal proliferating cells. We found that between the day of dosing and post-dosing, the control nestlings gained 49% more mass than the chlorpyrifos-dosed nestlings (mass gain control = +2.7 g; chlorpyrifos = +1.4 g). Chlorpyrifos also caused AChE activity to be lower post-dosing (AChE activity in controls = 3027.5 mU/ml; chlorpyrifos = 1632.2 mU/ml). Moreover, we will present how acute chlorpyrifos exposure may affect hippocampal cell proliferation throughout the nestling period. This project makes significant contributions to our understanding of how exposure to a commonly used pesticide affects the hippocampus of a migratory bird during a critical period of development, which is important because such effects could result in long-term changes on migratory behaviour and ultimately affect fitness.

**3.02.T-05 Characterizing possible relationships among chlorinated paraffins, ecological and endocrine measures in nestling peregrine falcons across the Canadian Great Lakes Basin.**

***Kim J. Fernie***[1]*, Cynthia A. de Wit*[2] *and Bo Yuan*[3]*, (1) Environment and Climate Change Canada, (2) Stockholm University, Sweden, (3) Norwegian University of Science and Technology (NTNU), Norway*

Chlorinated paraffins (CPs) are complex mixtures of polychlorinated *n*-alkanes that are categorized as short-chain (SCCPs, C10-13), medium-chain (MCCPs, C14-17), or long-chain CPs (LCCPs, C≥18). The differences in carbon chain length and chlorine content result in a wide range of physical-chemical properties that make them stable for a wide range of industrial applications, including lubricants, flame retardants, plasticizers, among other applications. They are produced in enormous quantities (2009: 1 M metric tons). Under the U.N. Stockholm Convention on Persistent Organic Pollutants, SCCPs were listed under Annex A (2017) and MCCPs and LCCPs are being reviewed. In Scandinavia, SCCPs, MCCPs and LCCPs were present in peregrine falcons (*Falco peregrinus*), with the highest concentrations found for LCCPs, which were the predominant CPs (55%) (Yuan et al. 2019). In the present study, we examine the CP profiles of nestling peregrine falcons sampled across the Canadian Great Lakes Basin predominantly in 2017. Our objectives are 1) to compare and contrast exposure levels and profiles of CPs in peregrine falcon nestlings, 2) to determine any effects of diet (inferred from stable carbon, nitrogen and sulfur isotopes), biology (age, sex), and geography (region) on CPs and thyroid hormones, and 3) to examine possible relationships between CP exposure, circulating thyroid hormones, body condition, and size of the nestlings. As with the Scandinavian adult peregrine falcons, the peregrine nestlings in central Canada had circulating concentrations of vSCCPs (C≤9), SCCPs, MCCPs that were dominated by LCCPs; comparatively, the Canadian birds had lower concentrations of SCCPs, similar MCCPs, and higher levels of LCCPs (47%), likely reflecting differences in age and tissues sampled and potentially differences in exposure to CPs between Scandinavia and central Canada. Urban peregrine chicks raised in southern Ontario had significantly higher concentrations of MCCPs and LCCPs than rural nestlings raised elsewhere in Ontario. Preliminary results suggest that circulating TT4 was significantly associated with circulating vSCCPs and SCCPs, and that SCCPs were associated with estimated thyroid gland function, consistent with previous research involving captive American kestrels, a species related to peregrine falcons in the Falconidae family.

**3.02.T-06 Pollution in The Coastal Wetlands of East Asia and The Decline in Migrating Shorebirds - Update on The COAST IMPACT Project**

***Veerle Jaspers***[1]*, Junjie Zhang*[1]*, Tobias Ross*[2]*, Hebo Peng*[3]*, Anne-Fleur Brand*[1]*, Alexandros Asimakopoulos*[1]*, Tomasz Ciesielski*[1]*, Courtney Waugh*[4]*, Dorte Herzke*[5]*, Giulia Poma*[6]*, Govindan Malarvannan*[6]*, Adrian Covaci*[6]*, Lei Wang*[7]*, Michelle Wille*[8] *and Marcel Klaassen*[2]*, (1) Norwegian University of Science and Technology (NTNU), Norway, (2) Deakin University, Australia, (3) University of Groningen, Netherlands, (4) Nord University, Norway, (5) NILU - Norwegian Institute for Air Research, Tromsø, Norway, (6 )University of Antwerp, Belgium, (7) Nankai University, China, (8) University of Sydney, Australia*

As part of the COAST IMPACT project, we investigate the influence of pollution and infectious diseases on shorebirds that migrate along the East Asian–Australasian Flyway (EAAF). Many EAAF shorebird populations are rapidly declining, the reasons for which are largely unknown. Climate change and habitat destruction are considered important contributors to these declines, but the role of pollution remaining mostly understudied. Pollutants may play an important role during migration, potentially affecting the availability and contamination of food on which shorebirds rely, and directly and indirectly (e.g. through immunomodulatory properties) impacting shorebird survival. COAST IMPACT assesses the combined impact of food availability, pollution, and disease on migrating shorebirds that use East Asian wetlands as stop-over sites and overwinter in Australia.

Sediment and macrobenthic samples from wetlands along the Chinese coast were analysed for metals/metalloids, persistent organic pollutants (POPs), per- and polyfluoroalkyl substances (PFASs), and other emerging contaminants (e.g. bisphenols and phtalate metabolites). Blood samples from shorebird species with differently reported population statuses (some in severe decline, others more stable) in Taiwan and Australia were analysed for metals/metalloids and PFASs. In addition, a Total Oxidisable Precursor Assay was performed to determine PFASs not amenable to standard analysis. POPs will also be analysed in tissues of shorebirds that were found dead in Australia. This will contribute to a robust evaluation of the total pollutant load in the birds along this EAAF and how it relates with avian influenza infection status of birds sampled in Australia.

Our initial data on sediment and macrobenthos indicates the Bohai Sea as a hotspot for contamination with PFASs and other emerging contaminants. In addition, the macrobenthos is declining in most of the studied stop-over sites in China. Shorebirds from Taiwan showed significantly higher PFASs concentrations than birds from Australia. The reported population status seems to be negatively correlated to total PFASs concentrations in the blood of the birds. Also, birds with high concentrations of PFASs had a higher chance of being infected with avian influenza. These relationships will be further investigated with new data on POPs and metals in the shorebirds and the results will be presented at the conference.

**3.02.T-07 Challenges in Interpreting Anticoagulant Rodenticide Exposure and Predicting Adverse Effects in Free-ranging Non-target Wildlife**

***Barnett A. Rattner***[1]*, Collin Eagles-Smith*[1]*, Garth Herring*[1]*, Julia Fonder*[2] *and Nimish Vyas*[1]*, (1) U.S. Geological Survey, (2) University of Minnesota*

Anticoagulant rodenticides (ARs) are used worldwide for control of vertebrate pests in urban and suburban settings, agriculture, and island restoration projects. From an ecological risk assessment standpoint, pesticide registrations are in part based on data from standardized laboratory toxicity tests, with field studies conducted when significant questions related to ecological risk remain unanswered. Standard acute oral toxicity tests markedly underestimate the hazard of first-generation ARs, and some second-generation ARs consistently fail ecological risk assessments, but remain in use because of the demand for effective rodent control. Field data offer significant improvements in realism relative to what can be observed in controlled laboratory registration studies that principally focus only on survival, growth and reproductive endpoints, although interpretation can be complicated. We present and compare AR exposure and effects data from field studies that included sublethal sampling of live animals (American kestrel *Falco sparverius*, red-tailed hawk *Buteo jamaicensis*, California condor *Gymnogyps californianus*) and passive tissue sampling from dead animals (various species of raptors in the U.S., lava lizard *Microlophus albemarlensis* in the Galápagos Islands). We address several questions: To what extent does sample integrity affect interpretation of exposure and toxicity? Does evidence of AR exposure and presence of residues equate to toxicity? Are there simple thresholds for AR residues in non-target wildlife? Is coagulopathy associated with other effects? Might AR residues move through the food webs to cause higher order effects? Are there additive or synergistic effects of co-exposure to multiple ARs and other contaminants (e.g., lead) that are commonly encountered by predatory and scavenging wildlife? While AR exposure can result in death of non-target wildlife, subtle and difficult to detect sublethal effects (e.g., coagulopathy, ptiloerection, immunotoxicity, behavioral aberrations) may occur that could have long-term consequences on survival. The role of field data in identifying AR exposure, adverse effects and sources of uncertainty to free-ranging wildlife will be discussed.

**3.02.T-08 Anticoagulant rodenticide toxicity in terrestrial raptors: Estimating potential mortality and impact on populations in North America and beyond**

*Veronica Silverthorn*[1]*,* ***John E. Elliott***[1]*, Peter Henrys*[2]*, Pierre Mineau*[3]*, Sandi Lee*[1]*, Victoria Bowes*[4]*, Tony Redford*[4]*, France Maisonneuve*[1]*, Joseph Okoniewski*[5]*, Christian Franson*[6] *and Stella McMillin*[7]*, (1) Environment and Climate Change Canada, (2) Center for Ecology and Hydrology, United Kingdom, (3) Pierre Mineau Consulting, Canada, (4) BC Ministry of Agriculture and Lands, Canada, (5) New York State*

*Department of Environmental Conservation, (6) National Wildlife Health Center, (7) California Department of Fish and Wildlife*
Anticoagulant rodenticides (ARs) have caused widespread contamination and poisoning of predators and scavengers, particularly terrestrial birds of prey. AR poisoning is diagnosed mainly on evidence of hemorrhage in conjunction with summed hepatic AR residues > 0.1 – 0.2 ug/g liver wet weight. However, simple summation of residues is confounded by variability in the potency and toxicokinetics of individual residues. Other factor also affect sensitivity of non-targets to ARs, including: exposure dose, timing and frequency of exposure; and, intra- and interspecific differences in sensitivity related to body condition, nutritional state, and other factors. There is, therefore, a need for compound and species-specific AR toxicity criteria to assess population-level effects on predatory birds. Previously we developed a novel approach based on probabilistic methods; however, with limited datasets and number of species, those assessments were limited in determining uncertainty around the probabilistic curves, along with other shortcomings, including assumed additivity, inability to analyze individual species and compounds, uneven sample size in the binary data set of birds classified as 1s (positive) or 0s (negative) for pathophysiological signs of anticoagulant rodenticide poisoning, as well as no assessment of how trauma cases bias results. In order to improve the approach we compiled a database of hepatic SGAR residues and post-mortem evaluations from 951 terrestrial raptor carcasses collected throughout Canada and the USA, 1989 – 2021, representing 26 species of mainly owls and hawks. We have developed species- and residue- specific probability curves to assess the toxicity of bromadiolone, brodifacoum, and difethialone and adjusted total SGARs using toxic equivalency factors from individual residue curves. To address the unequal sampling of 0s and 1s in the data, logistic regressions were run on Monte-Carlo simulated binary data.

## 3.02.P Field Studies for Reducing Uncertainty in Contaminant Exposure and Effects Assessments for Wildlife

### 3.02.P-Mo078 Heavy metal body burden in three species of ducks sampled from south-eastern mainland Australia

***Damien Nzabanita***[1]*, Jordan Hampton*[2]*, Jasmin Hufschmid*[2] *and Dayanthi Nugegoda*[1]*, (1) Royal Melbourne Institute of Technology, Australia, (2) The University of Melbourne, Australia*
Heavy metals are pollutants of increasing concern in aquatic systems. These contaminants are found in increasing concentrations in waterways since they are often found in industrial effluents and agricultural runoff. These chemicals bioaccumulate in the food web, potentially causing harmful effects on avian species such as ducks through processes ranging from eggshell thinning to mortality. In Australia, studies of heavy metal exposure levels in waterbirds are limited. The aim of this study was to determine the body burden of heavy metals in Pacific black ducks, *Anas superciliosa* (PBD), Grey teal, *Anas gracilis* (GT), and Australian wood duck, *Chenonetta jubata* (AWD). Breast feathers of ducks residing in the same location were collected, dried, and digested; and inductively coupled plasma-mass spectroscopy (ICP-MS) was used to measure heavy metal concentrations. The results showed that the mean level of all trace metals was significantly higher in GT than in the other two duck species. This was partially expected due to differences in feeding ecology, with GT being highly nomadic and dispersing more widely than the other two, more sedentary, species. This study provides important information on current trace metal levels in Australian freshwater systems.

### 3.02.P-Mo079 Of Whales and Men: A Multi-Year Study of Metals in Whales from the Gulf of Maine

***John P. Wise***[1]*, John P. Wise, Jr.*[1]*, Sandra S. Wise*[1]*, Jamie Young*[1]*, James Wise*[1]*, Catherine Wise*[2]*, Cynthia Browning*[3]*, Christopher Perkins*[4]*, Iain Kerr*[5] *and Tongzhang Zheng*[3]*, (1) University of Louisville, (2) Duke University, (3) Brown University, (4) University of Connecticut, (7)Ocean Alliance*
The Gulf of Maine is an important body of water for both humans and marine life, located on the Eastern seaboards of the United States and Canada. It is an important marine nursery, feeding ground and sanctuary, with over 3,000 marine species living there. The Gulf of Maine is also an area with heavy coastal development,

industry, and marine traffic, all of which contribute environmental chemicals to the Gulf resulting in chronic exposures of the people, animals and ecosystem that depend on the Gulf of Maine. The whales in the Gulf of Maine are a valuable sentinel species for pollution in the Gulf as they integrate all possible exposure routes and are key species integral to the health of the people, ecosystem and economies of the region. We collected whale skin biopsies for several years and measured the levels of 25 essential and non-essential metals in three whale species: humpback whales (*Megaptera novaeangliae*), fin whales (*Balaenoptera physalus*), and a minke whale (*Balaenoptera acutorostrata*). Overall, the general pattern of metal accumulation was consistent across species. As expected, we found the levels of the essential metals iron (Fe), magnesium (Mg) and zinc (Zn) to be among the highest levels for the metals we observed. Some metals of public health concern, aluminum (Al), nickel (Ni) and chromium (Cr), were also among the highest levels, while others, arsenic (As), cadmium (Cd), cobalt (Co), lead (Pb), mercury (Hg) and uranium (U) were comparatively low. Comparisons with similar species from other regions indicate humpback whales have levels of genotoxic metals (i.e. DNA damaging) that are among the highest in the world. These data indicate metal exposure may be a significant health concern for the Gulf of Maine ecosystem. This work was supported by the National Institute of Environmental Health Sciences [R01ES016893 and R35ES032876 to JPWSr.].

**3.02.P-Mo080 Using a qPCR Array to Measure Hepatic mRNA Expression in Tandem With Contaminant Biomonitoring to Assess Potential Effects in Seabird Embryos**

***Mason D. King*[1]*, John Elliott*[2]*, Doug Crump*[2]*, Vicki Marlatt*[1] *and Tony Williams*[1]*, (1) Simon Fraser University, Canada, (2) Environment and Climate Change Canada*

Birds can bioaccumulate persistent contaminants, and maternal transfer to eggs may expose sensitive embryos to high concentrations. However, using tissue residue concentrations alone to infer whether adverse effects are occuring suffers from uncertainty and is a major limitation in many biomonitoring programs. Furthermore, efficient and sensitive biomarkers to assess effects caused by contaminants remain limited or unvalidated in many avian wildlife sentinel species, although mRNA expression-based methods are promising. Therefore, we evaluated spatial and correlational relationships between whole embryo contaminant concentrations (total mercury, 20 organochlorine pesticides, 17 perfluoroalkyl substances, 35 polychlorinated biphenyls, and 22 halogenated flame retardants) and mRNA expression of 24 toxicologically relevant genes in embryonic liver tissue from a Pacific Ocean Alcidae seabird, the rhinoceros auklet (*Cerorhinca monocerata*). Fresh, unincubated eggs were collected and artificially incubated in the lab. Pre-hatch embryos were sampled for hepatic tissue and RNA was extracted for qPCR array analysis ($2^{-\Delta Cq}$). The remaining embryo carcass was analyzed for total mercury and organic contaminants. Contaminant concentration data and qPCR normalized gene expression data were analysed with a combination of linear models and multivariate approaches (PCA, correlation matrix, cluster analysis). Results suggest an association between embryonic contaminants and mRNA expression of certain genes. For instance, the gene Sepp1, which encodes selenoprotein, was significantly positively correlated with whole embryo total mercury concentration. This study provides valuable baseline data on contaminant concentrations in wild seabirds in northwestern North America and examines the utility of gene expression approaches for use in contaminant biomonitoring. This type of approach could help assess potential effects of pollution in free living wildlife at contaminated sites where remediation is under consideration, for instance.

**3.02.P-Mo081 Development of a novel in situ bioassay system for assessing oil spill toxicity in aquatic organisms**

***Rachel R. Leads*[1]*, Brianne Soulen*[1]*, Alexis Khursigara*[1]*, Michelle Krasnec*[2]*, Oscar Garcia*[3]*, Jeffrey Morris*[2] *and Aaron Roberts*[1]*, (1) University of North Texas, (2) Abt Associates, (3) WaterMapping, LLC*

An estimated 53% of oil enters the aquatic environment due to anthropogenic activity relating to the extraction, transportation, and utilization of petroleum hydrocarbons. These sources can include stormwater runoff, wastewater effluent, boat traffic, offshore oil operations, and accidental oil spills. Oil spills can be particularly damaging to aquatic systems because they typically release large volumes of oil into ecosystems that are not

adapted to significant hydrocarbon exposure. Laboratory studies following large scale oil spills have provided important information on the impacts and physiology of oil toxicity in aquatic organisms; however, very few studies have characterized toxicity in situ following an oil spill event. In situ testing is critical for understanding the effects of oil in variable, environmentally relevant conditions. This is particularly important because oil toxicity can be significantly modified by abiotic factors such as ultraviolet light, natural organic matter, weathering, and dissolved oxygen which may not always be incorporated during laboratory testing. Therefore, the present study developed a novel in situ bioassay system for assessing oil spill toxicity in aquatic organisms. The flow-through system consists of water-tight housing containing a battery and peristaltic pumps, a buoyant base, and test chambers through which exposure water is pumped. The system is portable and modular, allowing for rapid deployment and data collection during oil spill response operations. Flow-through laboratory and field testing of early life stage fish and invertebrates demonstrated that the system can successfully be used for testing of aquatic organisms. This research provides an accessible system for in situ toxicity testing and can contribute to our understanding of oil spill impacts under environmentally relevant conditions.

**3.02.P-Mo082 Associations between Organic Contaminants and Vitamins A and E in the Plasma of Great Lakes Pre-fledgling Double-Crested Cormorants**

***Keith Grasman*[1]**, *Stephanie Oosterhouse[1], Abigail Triemstra[1], Mandy Annis[2], Carly Eakin[2], Donald Tillitt[3] and James Ludwig, (1) Calvin University, (2) U.S. Fish and Wildlife Service, (3) U.S. Geological Survey*

Vitamin A plays essential roles in vision, immunity, and development. Vitamin E is an antioxidant and supports immune function. Concentrations of vitamins in tissues are influenced by diet, tissue type, age, cellular/physiological metabolism, health status, and contaminants. Dioxin-like contaminants such as planar PCBs have been associated with reduced vitamin A in wild and experimental animals, such as Great Lakes waterbirds. Vitamin E interacts with contaminants like PAHs and dioxins that induce oxidative stress. The objectives of this study were to determine whether plasma concentrations of vitamins A and E differed in pre-fledgling double-crested cormorants (*Phalacrocorax auritus*) at or near highly contaminated Great Lakes Areas of Concern (AOCs) compared to less contaminated sites and whether vitamin concentrations were associated with organic contaminants. Across the Great Lakes, blood samples were collected from 355 chicks at 16 colonies in 2016 and 15 colonies in 2017. When colonies in or near AOCs were combined, plasma vitamin A was 38-42% lower and vitamin E 45-49% lower compared to sites outside of or in recovering AOCs (t-test P values<0.0001 both years). Contaminant concentrations in plasma pooled by site ranged from 4.0–91.9 ng/g for sum PCBs, 0.18–4.6 pg/g for planar PCB TEQs (2016), 1.6–11.9 ng/g for DDE, 0–2970 ng/g for sum PBDEs, and 0.12–4.5 ng/g for PAHs. Vitamin A had moderate negative association with planar PCB TEQs (P<0.013) and no association with other contaminants. Vitamin E showed strong negative associations with PCBs in both years, planar PCB TEQs and PAHs in 2016 (P values<0.0001); moderate negative associations with PAHs (2017, P<0.029) and DDE (both years, P values<0.039); and no associations with sum PBDEs. Causation criteria, including replication (multiple years and sites), biological coherence (known mechanism for vitamin A, previous studies for vitamin E), and strength of association (extremely low p values, magnitude of dose response), suggested contaminants can alter vitamin homeostasis. Vitamin A was associated with planar PCBs and vitamin E was associated with PCBs, PAHs, and to a lesser extent DDE in young cormorants at and near highly contaminated AOCs in the Great Lakes.

**3.02.P-Mo083 Neural Correlates of Spatial Behavior in Relation to Contaminant Concentrations in Free-Ranging Gulls**

***Anna Lippold*[1]**, *Jonathan Verreault[2] and Mélanie Guigueno[1], (1) McGill University, Canada, (2) University of Quebec at Montreal, Canada*

Many tasks of an animal's life, such as foraging or finding and remembering places for important life-history stages (e.g. mating or breeding) heavily rely on spatial ability, a combination of spatial memory and navigation. The brain region most heavily involved in both is the hippocampus. It has been shown that individuals or species that rely more heavily on spatial ability than conspecifics or closely related species also have a larger hippocampus and higher levels of neurogenesis (i.e., generation of new neurons). As such a central organ for ecologically relevant behaviors, the brain and with it the hippocampus are vulnerable to threats such as environmental contaminant exposure. In fact, adverse effects of some environmental contaminants have been found on brain regions involved in song and mating, however, effects of contaminants on spatial memory or the hippocampus have received limited attention.

The objective of this study is to quantify neural correlates of spatial behavior, such as hippocampal volume and the level of neurogenesis, in relation to spatial behavior strategies and brain contaminant concentrations in ring-billed gulls (*Larus delawarensis*) breeding in a colony close to the heavily populated city of Montréal (Québec, Canada). Individuals from this particular population of ring-billed gulls are exposed to remarkably high levels of certain contaminants, such as halogenated flame retardants and persistent organic pollutants; and additionally show considerable variation in spatial behavior strategies. We will present results on hippocampal volume and levels of neurogenesis in relation to measures of spatial ability, such as home range size, number of feeding locations, and path straightness; and how both spatial ability and its neural correlates are related to brain concentrations of environmentally ubiquitous halogenated flame retardants and persistent organic pollutants.

**3.02.P-Mo084 Associations between Organic Contaminants and Thiamine in the Blood of Pre-Fledging Great Lakes Double-Crested Cormorants**

***Keith Grasman***[1]*, Abigail Triemstra*[1]*, Stephanie Oosterhouse*[1]*, Mandy Annis*[2]*, Carly Eakin*[2]*, Donald Tillitt*[3] *and James Ludwig, (1) Calvin University, (2) U.S. Fish and Wildlife Service, (3) U.S. Geological Survey,*

Thiamine (vitamin B1) plays essential roles in energy metabolism, growth, development, immunity, and antioxidant defenses. Vitamin tissue concentrations can be influenced by tissue type, diet, age, health status, metabolism, and contaminants. Thiamine deficiency has been identified in Great Lakes salmonids and fish-eating birds in other areas. This study's objectives were to determine whether whole blood concentrations of thiamine, thiamine diphosphate (TDP), thiamine monophosphate (TMP), and these vitamers' sum (sum T) differed in pre-fledgling double-crested cormorants (*Phalacrocorax auritus*) at or near Great Lakes Areas of Concern (AOCs) compared to less contaminated sites and whether thiamine concentrations were associated with organic contaminants. Blood samples were collected from 361 cormorant chicks in 2016 and 2017 from 19 colonies across all five Great Lakes. At colonies in or near AOCs, thiamine (49-70%), TDP (24-36%), TMP (42-53%), and sum T (39-58%) were lower compared to less contaminated sites outside of AOCs or in recovered AOCs (t-test P values<0.0001 except P<0.0011 for TDP in 2016). Contaminant concentrations in plasma pooled by site ranged from 4.0–91.9 ng/g for sum PCBs, 0.18–4.6 pg/g for PCB TEQs (2016 only), 1.6–11.9 ng/g for DDE, 0–2970 ng/g for sum PBDEs, and 0.12–4.5 ng/g for PAHs. Plasma DDE had strong negative associations with all thiamine variables in both years (Jonckheere trend tests P values<0.01 except P<.032 for thiamine in 2017). Sum PCBs (both years) and PCB TEQs (2016) had strong negative associations with thiamine, TMP, and sum T (P values<0.001). TDP had negative associations with PCBs in 2017 (P<0.004). PAHs had strong negative associations with thiamine in both years (P values<0.0001) and with TDP, TMP, and sum T in 2017 (P values<0.002). PBDEs had significant negative associations with all thiamine variables in 2016 (P values<0.039). Strength of associations between multiple contaminant and thiamine variables and consistency upon replication (spatially and temporally) indicate organic contaminants play a role in influencing thiamine concentrations in young cormorants. No ecological or dietary factors were apparent as strong explanations for the differences in vitamin concentrations among colonies.

**3.02.P-Mo085 Pesticides Detected in Honey Bee Hive Pollen in North-Central Oklahoma and Potential Effects on Hive Health**

***Carrie C. Klase****, Jason B. Belden and Kristen Baum, Oklahoma State University*

Honey bees (*Apis mellifera*) are key pollinators of a variety of crop and wildflower species. With declines in the survival of commercial hives, understanding the variables that impact honey bee hive health is critical to the agricultural industry. Managed honey bee hives are frequently placed on or near crop fields to provide pollination services, which increases the risk of exposure to pesticides. We placed three hives at 24 study sites (12 sites in 2019, four near each land use type of canola crop, wheat crop, and grasslands, and then repeated at different site locations in 2020) in north-central Oklahoma. Hives remained at the sites from late March to early October, and samples were collected three times during that time period. During each sampling period, we collected samples to assess pesticide presence in pollen from hive pollen traps and pesticide residue on nearby crops and wildflowers. To assess hive health, we measured hive weight changes, *Varroa* mite loads, and the presence of *Nosema* spores in each hive during each sampling period. Our objective was to determine the extent of pesticide exposure during our sampling periods and to evaluate the possible relationship between landscape, pesticide exposure, and honey bee health across three land use types. Within the 33 hive pollen samples that were collected, a total of 20 pesticides were detected, with seven pesticides detected at trace levels only and six detected in only a single sample. Of the seven pesticides detected above trace levels in multiple samples, the majority were fungicides. Almost all pesticides detected in hive pollen were below LC50 levels. No significant relationship was found between land use type and average number of pesticides detected in hive pollen, mite presence, and long-term weight changes. However, a significant relationship was detected between year, pesticides, and hive health. These results indicate that other variables, such as contact exposure to pesticides through plant surface residues, nutrition, or weather, may be better predictors of hive health than land use type and pesticides in pollen collected by bees. Samples for pesticide contact exposure and *Nosema* spore presence are still undergoing analysis. Results of this research can be used to better understand the effects of pesticides on honey bee hives at realistic exposure levels.

## 3.02.V Field Studies for Reducing Uncertainty in Contaminant Exposure and Effects Assessments for Wildlife

### 3.02.V-01 The Effects of Ditch Management in Agroecosystems on Embryonic and Tadpole Survival, Growth and Development of Northern Leopard Frogs (Lithobates pipiens)

*Amber Dyck[1], **Stacey A. Robinson**[2], Sarah Young[2], Justin Renaud[3], Lyne Sabourin[3], David Lapen[3] and Frances Pick[1], (1) University of Ottawa, Canada, (2) Environment and Climate Change Canada, (3) Agriculture and Agri-Food Canada (AAFC)*

Agricultural drainage ditches drain excess water from fields while providing ecosystem services and habitat for wildlife. In regions where they occur, these ditches can occupy thousands of kilometers. Ditch management practices such as vegetation clearing/brushing and dredging can influence these ecosystem services and ecological functions. Our objective was to determine how ditch management practices affect the survival, growth and development of northern leopard frogs (*Lithobates pipiens*) during their aquatic life stages. Using *in situ* cages with embryos and tadpoles, we conducted three field studies over two years in vegetated/unmanaged (i.e. no clearing/brushing or dredging) and recently cleared/brushed/dredged (i.e. treeless, then dredged) drainage ditches in a river basin in Eastern Ontario, Canada and measured nutrients, pesticides and physiochemical water quality properties. At the recently cleared/brushed/dredged sites, we found high concentrations of glyphosate, but lower concentrations of nutrients, atrazine, and total neonicotinoids. These sites typically also had higher temperature, dissolved oxygen, and turbidity levels. We observed premature hatching at one of the vegetated/unmanaged sites, but overall did not detect a significant difference among ditch management practices in the survival, hatching success or development of embryos. However, we found tadpoles at the cleared/brushed/dredged sites were significantly more developed and had larger body sizes, likely because of higher water temperatures imposed by reducing shading effects by trees. Our results suggest physical and chemical differences among ditch management practices but no obvious detrimental effects on the survival, growth or development of northern leopard frog embryos or tadpoles. Additional work is required on the effect of temperature, within drainage ditches with longer exposure times, as the long-term consequences on amphibian populations remains unclear.

## 3.03 Non-invasive Techniques to Biomonitor Exposure and/or Effects from Anthropogenic Pollutants in Wildlife

### 3.03.T-01 Associations Between Organohalogen Contaminants and Thyroid Hormones in Skin of the Endangered St. Lawrence Estuary Beluga

***Valérie Jolicoeur***[1]*, Magali Houde*[2]*, Robert Michaud*[3] *and Jonathan Verreault*[1]*, (1) University of Quebec at Montreal, Canada, (2) Environment and Climate Change Canada, (3) Group for Research and Education on Marine Mammals, Canada*

The St. Lawrence Estuary (SLE) beluga (*Delphinapterus leucas*) population is highly exposed to an array of organohalogen contaminants that were identified to be one major cause for the non-recovery of this endangered and declining population. In the last decade, an increasing number of parturition-associated complications and calf mortality has been reported in this population. It was suggested that elevated exposure to certain organohalogens (e.g., polybrominated diphenyl ethers [PBDEs]) may play a role in this phenomenon by decreasing thyroid hormone levels (i.e., hypothyroidism). The objective of the present study was to investigate the associations between concentrations of priority organohalogen contaminants and thyroid hormones in skin of adult male and female SLE belugas. Because plasma could not be collected in SLE belugas for ethical reasons, skin biopsy was used as a novel matrix to analyze organohalogens and thyroid hormones in this population. A total 40 skin biopsy samples were collected and analyzed for halogenated flame retardants (including PBDEs), polychlorinated biphenyls and organochlorine pesticides, as well as thyroid hormones (triiodothyronine [$T_3$] and thyroxine [$T_4$]) and their deiodinated products (reverse $T_3$ and 3,5-diiodothyronine). Chemical analysis was carried out using a gas chromatograph coupled to a single quadrupole mass spectrometer, while thyroid hormones were analyzed by an ultra-performance liquid chromatograph coupled to a mass spectrometer in multiple reactions monitoring mode (UPLC-MRM/MS). This novel UPLC-MRM/MS-based method allowed for the identification and quantification of these thyroid hormones and their deiodinated products in skin, showing that this matrix may represent a promising alternative for plasma for the evaluation of thyroid status in endangered cetacean populations. Comparison of linear models (Akaike information criterion) showed that the best predictor for the $T_4/T_3$ level ratio in SLE beluga skin was the sex of the individual. In males, $T_3$ concentrations in skin were positively correlated with $S_{35}$PBDE concentrations. No association was found for other organohalogens. These results suggest that thyroid hormone levels could be disrupted by PBDE exposure, but do not support the hypothesis of decreased thyroid hormone levels associated with contaminant exposure in St. Lawrence belugas, potentially leading to hypothyroidism.

### 3.03.T-02 Using seabird eggs to monitor spatial and temporal trends of legacy POPs in the Northeast Pacific, 1968 to 2019

***Robert Kesic***[1]*, John E. Elliott*[2]*, Kyle Elliott*[3] *and Sandi Lee*[2]*, (1) Simon Fraser University, Canada, (2) Environment and Climate Change Canada, Canada, (3) McGill University, Canada*

Seabirds are useful indicators for monitoring legacy POP levels in marine ecosystems because they feed at relatively high trophic levels, suitably integrating contaminant exposure across space, time, and communities. For decades, seabird eggs have been used as a non-invasive sampling matrix to monitor contaminant exposure and biomagnification processes in marine food webs because they are: 1) easy to identify and collect, 2) easy to homogenize and analyze, and 3) representative of the contaminant burden in the female adult at the time of laying. Here, we compared spatial and temporal trends of organochlorine insecticides and PCBs, as well as stable isotopes of $\delta^{13}$C and $\delta^{15}$N, in the eggs of five seabird species (double-crested cormorants, pelagic cormorants, rhinoceros auklets, ancient murrelets, and Leach's storm-petrels) breeding off the Pacific coast of British Columbia, Canada from 1968 to 2019. The primary OC insecticides detected in the eggs of all species and sampling years were ΣDDT (mostly *p,p*'-DDE), ΣHCH (mostly β-HCH), ΣCHLOR (mostly oxychlordane), and ΣCBz (mostly HCB). The majority of legacy POPs are either significantly declining (e.g. *p,p*'-DDE, HCB, heptachlor epoxide, ΣPCBs) or showing no directional change over time (e.g. ΣMirex) in the eggs of our seabird species. Compounds such as α-HCH, γ-HCH, *cis*-chlordane, *trans*-chlordane, octachlorostyrene, *p,p*'-DDT, *p,p*'-DDD, and dieldrin also showed evidence of downward trends, largely influenced by non-detect values (i.e., < MDL) during more recent sampling periods. Increasing trends were observed for oxychlordane and β-HCH in the eggs of some species; however, concentrations of these contaminants eventually returned to early 2000 levels by the end of the study period. Our most parsimonious description describing temporal trends in POP concentrations was one that included a quadratic effect of time and an effect for location, though other models were also equivalent based on the $\Delta AIC_C$. Differences for egg $\delta^{13}C$ and $\delta^{15}N$ values were also found between species and colonies, consistent with different trophic relationships, foraging habitats, and dietary uptake of contaminants during the breeding season.

**3.03.T-03 Monitoring Flame Retardants in Air from the Back of a Gull: The Landfill Effect**
***Jonathan Verreault**[1], Anaïs Kerric[1], Manon Sorais[1], Marc Mazerolle[2] and Jean-François Giroux[1], (1) University of Quebec at Montreal, Canada, (2) Laval University, Canada*
Elevated levels of halogenated flame retardants (HFRs) have been reported in breeding ring-billed gulls (*Larus delawarensis*) from the Montreal area (QC, Canada). Previous studies from our lab demonstrated that atmospheric exposure to PBDEs determined using miniature passive air samplers (PAS) carried by these gulls was highest in individuals foraging in landfills. Exposure to HFRs can occur through multiple pathways including diet, inhalation, and ingestion during preening of HFRs adhering on their feathers. However, very few studies have explored associations between air HFR levels and gull exposure within landfills, as well as the contribution of the different exposure pathways in birds. The present study aimed to investigate (1) the distribution of HFRs in air samples collected within one major landfill in the Montreal area that is frequently visited by ring-billed gulls, and (2) the contribution of different HFR exposure pathways in these gulls. Miniaturized PASs and active air samplers (AASs) were deployed in six different areas within this landfill for 34 days. Gulls were equipped on their back with a similar PAS along with a GPS datalogger for ten days. PBDEs and other HFRs were analyzed in air samples, as well as in plasma, lung, feather surface, preen oil, liver, and stomach content of the tracked gulls. Results showed that elevated concentrations of PBDEs were equally distributed within different sections of the landfill. In addition, the tracking of gulls confirmed that landfills represent a major emission source of PBDEs for birds visiting a landfill at least once, with significantly greater concentrations of BDE-47 in their PASs. We further showed, using structural equation models, that atmospheric exposure of these urban-adapted gulls to PBDEs could occur through both inhalation (air) and ingestion (feather preening and stomach content). Lung concentrations of BDE-28 was directly influenced by air levels in PASs. We also demonstrated that PBDE concentrations in liver increased with those in lungs, feather surface, and stomach contents. We conclude that atmospheric exposure to HFRs should not be underestimated compared to the traditional dietary exposure pathway in urban-adapted, omnivorous birds.

**3.03.T-05 Development of a site-specific non-invasive biomonitoring tool for determining source attribution and estimating bioaccessible Pb in Tundra Swans at the Bunker Hill Superfund Site**
***Todd Luxton**[1], Mark Jankowski[1], Chris Eckley[1], Matthew Noerpel[1], Anna M. Wade[2] and Richard Wilkin[1], (1) U.S. Environmental Protection Agency, (2) Oak Ridge Institute for Science & Education at US EPA, Cincinnati*
The Bunker Hill Superfund Site in northern Idaho and eastern Washington is one of the largest historical mining districts in the world. Tailings and smelting waste from historical mining caused widespread contamination of lead (Pb) across the lower basin of the Coeur d'Alene River in the wetlands, side lakes, and floodplains. Elevated Pb levels in these wetlands have adversely impacted the health of tundra swans (*Cygnus Columbianus*) that feed in the lower basin for several weeks each year during their springtime northern migration. While foraging for aquatic plants such as the Wapato (*Sagittaria latifolia*; water potato), the swans ingest contaminated sediment that can result in acute Pb toxicity. The goal of the current study is to develop a non-invasive biomonitoring tool to determine the source and bioaccessibility of Pb in tundra swans exposed in the lower basin. Samples of swan blood, feces, and co-located sediments were collected in areas of known contamination within the basin and a reference site outside the basin.  Avian species and relative abundance of plants consumed were identified by metagenomic analysis of fecal DNA. Differences in the ratios of Pb stable

isotopes in blood, sediment and feces were used to determine the source of Pb. The bioavailability of Pb was established by first comparing the concentration of Pb present in the blood with Pb concentrations in paired feces samples and sediments located near capture. X-ray absorption spectroscopy (XAS) was used to further understand Pb bioavailability during ingestion by comparing the speciation of Pb in the sediment with the speciation of Pb in the feces. The results strongly suggest the source of Pb the swans are exposed to originates from the Bunker Hill Superfund Site. The high concentration of Pb present in the blood and a quantifiable reduction in the concentration of lead present in the feces compared to the sediments suggests the Pb at the Bunker Hill site is bioavailable. XAS data suggests that Pb complexed with organic matter is the most bioavailable form of Pb present. This baseline data set may be used to determine Pb source attribution throughout the basin in tundra swans through non-invasive analysis of fecal matter. Further, our study suggests changes in the bioavailability of Pb in sediments over time can be monitored through the ratio of Pb in the fecal samples and co-located sediment as well as by changes in the Pb speciation pre and post ingestion.

**3.03.T-06 Trace Elements in Shark Blood: Can Blood Be Used for Non-lethal Biomonitoring?**
***Julia Smith**[1], Jason van de Merwe[2], Liesbeth Weijs[2], Frederic Leusch[3] and William Bennett[2], (1) Griffith University, Australia, (2) Coastal and Marine Research Centre, Australia, (3) Australian Rivers Institute, Australia*
Sharks play a crucial predatory role in aquatic ecosystems, but many species and populations are highly vulnerable to a wide range of anthropogenic stressors. While the largest threat includes fishing mortality, sharks may also be affected by pollution. Measuring non-essential (i.e. mercury) and essential trace elements (i.e. selenium) supports the understanding of sharks' overall health by documenting their trophic ecology and exposure to contaminants. Many studies measuring trace elements in sharks perform analyses on internal tissues from deceased specimens, such as muscle and liver. However, these samples are not always obtainable for research purposes, especially from live sharks. Alternatively, blood samples can be routinely collected from sharks that are captured and released alive for other research projects. Although a few recent studies have reported trace element concentrations in shark blood, there are still many unanswered questions on whether blood can be used to meaningfully monitor trace elements in sharks. The objectives of this study were to assess if multiple essential and non-essential trace elements: 1) differ between blood compartments (whole blood, plasma, and serum); 2) are impacted by different anticoagulants used for whole blood and plasma; 3) differ in blood samples taken before and after euthanasia; and 4) exhibit relationships between paired muscle, liver, and blood samples. In addition, baseline concentrations in blood are reported for bull, *Carcharhinus leucas* ($n = 19$), and tiger sharks, *Galeocerdo cuvier* ($n = 4$). Samples were collected opportunistically in collaboration with the Queensland Shark Control Program (euthanized animals) or from specific research field trips. Microwave-assisted digestion followed by inductively coupled plasma – mass spectrometry (ICP-MS) was used to measure over 40 elements in collected tissues, including chromium, manganese, iron, cobalt, copper, arsenic, selenium, and mercury. This research will assist in standardizing future sample collection protocols for trace element analysis in shark blood while also providing baseline concentrations to better assess shark health and their exposure to contaminants.

**3.03.T-07 Decentralizing ecosystem toxicology to evaluate mercury burden across neotropical biota in the Peruvian Amazon**
***Caroline Renee Moore**[1], David C. Evers[2], Pamela Sánchez Vendizú[3], Jhakelin Gloria Reyes Vasquez[3], Thalía Araceli Silvestre Espejo[3], Gideon Erkenswick Watsa[4] and Mrinalini Erkenswick Watsa[1], (1) San Diego Zoo Wildlife Alliance, (2) Biodiversity Research Institute, (3) Conservación Amazónica, Peru, (4) Washington University in St. Louis*
The Madre de Dios region of the Peruvian Amazon is heavily mined outside of protected conservation concessions and national parks, resulting in about 20% of global gold production. Due to the fine size of the gold, mercury is used to bind the gold and separate it from the river silt as an amalgam. The mercury is then burned off, resulting in mercury contamination of land, water, and air, leading to impacted human health. Yet

there is very little known about the impact on wildlife in the region, a region with one of the highest levels of biodiversity in the world. A recent study in the region demonstrated intact forests next to artisanal and small-scale gold mining (ASGM) sequester mercury at levels comparable to industrial cities, and bird feathers from forests proximal to ASGM have higher mercury levels compared to similar species distanced from ASGM in the neighboring protected national park. This studied region, proximal to ASGM and a 4-hour boat ride up the Amazon River from the nearest town, is called the Los Amigos Conservation Concession. At the associated Eatacion Biologica Rio Los Amigos, a 12-year tamarin mark-recapture program has recently been expanded through the In Situ Laboratories (ISL) initiative to include a decentralized, cost-effective, and sustainable molecular laboratory to perform biodiversity monitoring, pathogen disease surveillance, and toxicology research by leveraging advances in conservation technology. This presentation will discuss the development of this Amazonian ISL Wildlife Conservation Laboratory and how the current mark-recapture program, which collects non- and minimally-invasive tissue samples from roughly one thousand animals a field season (birds, bats, small and medium sized mammals, non-human primates), is resulting in a database of hair and feather mercury levels across biota per year that can be used to better understand mercury movement through neotropical wildlife and the intersection of disease and mercury exposure. Different techniques and methods explored for mercury testing will be discussed, along with mercury data relevant per species group. Additionally, our layered data approach will be outlined, which will be used to understand how animal heath, movement, genetics, and pathogen burden can be studied to understand whole ecosystems in relation to mercury exposure, opening the door to field-based, community-run toxicology science.

**3.03.T-08 Local vs Global: Identifying Sources of Polychlorinated Biphenyl Contaminants Affecting the Health of Southern Resident Killer Whales Using Passive Sampling and Chemical Activity**

***Kelsey Lee*** *and Frank Gobas, Simon Fraser University, Canada*

Southern Resident killer whales are a genetically, socially distinct population of killer whales that frequently inhabit the waters of the Salish Sea. Their population is in decline and has been registered on the Species at Risk Act in Canada as well as the Endangered Species Act in the US. The three main anthropogenic threats identified include reduced quantity and quality of their main food source, Chinook salmon, as well as acoustic and physical disturbance from vessels and exposure to environmental contaminants. Of the contaminants the whales are being exposed to, those of greatest concern include polychlorinated biphenyls (PCBs), a legacy persistent organic pollutant. Although the use and import of PCBs has been banned in North America since the 1970s, recent studies suggest the levels of PCBs in the environment and the whales themselves indicate that they are still being released today. The objective of this study is to determine the sources of PCBs to the killer whales. To address this objective, we employed the use of passive sampling of air and water to measure PCBs in the Salish Sea. These environmental concentrations were combined with concentration data of PCBs in sediments, salmon, and killer whales in a chemical activity and fugacity analysis and presented in a chemical heat map. The results indicate that local sources are primarily responsible for the current body burden of PCBs in the Southern Resident killer whales. Long range atmospheric transport of PCBs to the Salish Sea is a minor source. The analysis identifies a number of key local sources of PCBs to air, water, and sediments that can be controlled. The study illustrates the application of passive sampling, chemical activity and fugacity analysis, and chemical heat mapping as a useful approach to link sources of PCBs to the body burdens of PCBs in salmon and killer whales. The results provide opportunities for pollution abatement efforts to reduce or prevent further release of contaminants into the environment.

## 3.03.P Non-invasive Techniques to Biomonitor Exposure and/or Effects from Anthropogenic Pollutants in Wildlife

**3.03.P-Tu065 Biomonitoring Heavy Metal in European Hedgehog (Erinaceus europaeus) Spines: Influence of Age**

*Javier García-Muñoz[1],* ***Annalisa Zaccaroni***[2]*, Salome Martinez Morcillo[1], Francisco Soler Rodriguez[1], Maria*

*Prado Míguez-Santiyán[1], Luis Eusebio Fidalgo Alvarez[3], Ana Lopez Beceiro[3] and Marcos Perez Lopez[1], (1) University of Extremadura, Spain, (2) University of Bologna, Italy, (3) University of Santiago de Compostela, Spain*
Heavy metals are potential harmful elements, widely released by anthropogenic sources, being highly persistent and toxic in the ecosystems. In this sense, small mammals are suitable biomonitors of metal pollution due to their relevant position in the trophic web and high metabolic rate, being very sensitive to accumulate toxic metals. Non-invasive tissues constitute a useful matrix from a ethical point of view, giving representative results in term of population and/or individual chronic exposure. With these considerations, the purpose of this study was to determine the levels of Cd, Hg, Pb, Zn and As in European hedgehog (*Erinaceous europaeus*), using spines as biomonitoring tissue, also assessing the influence of age on the accumulation of these elements.

Hedgehog spines (n = 40) were collected at different Wildlife Recovery Centers from Galicia (NW Spain) and sent to the Veterinary Faculty of Cáceres (SW Spain) for chemical analysis. The age of the animal was estimated by their general size, as well as their dental development and degree of sexual maturity (31 adults and 9 young). Spines were dried overnight and digested using a microwave automatic digester, and metal quantification was carried out by means of ICP-MS, with the adequate quality controls. The obtained data were statistically analyzed using GraphPad Prism 9. The results were expressed in terms of dry weight.

The highest mean concentration was quantified for Zn (83.1 mg/kg), followed by Hg (6060 µg/kg), Pb and As (mean values of 486.8 and 284.6 µg/kg), and the mean minimum value corresponded to Cd (15.71 µg/kg). No statistically relevant correlations among metal levels in hedgehog spines were observed, except for Cd and Pb ($r=0.689$, $p<0.001$), in this case showing similar toxicokinetics of both metals. Regarding the effect of age, its influence was statistically significant ($p<0.05$) for Zn, its concentrations being higher in adults (84.41 µg/kg) when compared to young (78.38 µg/kg). A similar pattern was observed for the remaining metals, although these differences were not statistically significant ($p>0.05$). In conclusion, spines can be used as a suitable non-invasive tissue in future biomonitoring programs using hedgehog as sentinels. Moreover, the influence of age can represent a relevant factor to be considered when quantifying metal accumulation.

**3.03.P-Tu066 Application of Environmental DNA Metabarcoding to Assess Fish Biodiversity at a Legacy Polycyclic Aromatic Hydrocarbon-Contaminated Site**

*Brittney Graham, James Feller and **Roman Lanno**, The Ohio State University*
Environmental DNA (eDNA) metabarcoding has emerged as a quick and cost-effective tool for monitoring biodiversity in aquatic systems. The high throughput nature of this technology and improved sample efficiency overall makes eDNA an ideal alternative to traditional, more labor-intensive environmental survey methods. eDNA metabarcoding can be used to simultaneously evaluate the effects of environmental stressors on the biodiversity of a wide variety of taxa. Polycyclic aromatic hydrocarbons (PAHs), a byproduct of fuel combustion, industrial manufacturing, coal-tar creosote operations, etc., are persistent carcinogens and frequently found at contaminated sites. To evaluate the utility of eDNA metabarcoding in assessing the possible effects of PAHs on fish biodiversity, a study was conducted of the Little Scioto River near Marion, OH, a known creosote contamination site dating back to the 1800s. Extensive chemical monitoring and a series of traditional biological surveys, in addition to several remediation efforts by the US EPA, have taken place at this site over the past several decades. The objective of this study was to use eDNA metabarcoding to evaluate fish biodiversity of an upstream reference site and compare this with biodiversity at the PAH remediation site, and a downstream site to assess the impact of PAH contamination and the efficacy of remediation efforts (2002-2006) 15 years later. Water samples (1L) were taken in triplicate at 3 sites along the Little Scioto River, in addition to a secondary reference site along the Scioto River, upstream of its confluence with the Little Scioto River. The triplicate samples were pooled from each site (~1/3 L each) and filtered through 1.2um cellulose nitrate filters. After filtration, eDNA was extracted from these filters via the Qiagen DNAeasy PowerSoil kit, and samples

were sent for sequencing using a 12S MiFish metabarcoding approach. The results of this study will be compared to other biota surveys conducted at the site in previous years.

### 3.03.P-Tu067 Measuring Biodiversity and Ecosystem Function in Mining-Impacted Streams using Environmental DNA (eDNA) Metabarcoding

*James Feller, Kaleigh O'Reilly and **Roman Lanno**, The Ohio State University*

There is a pressing need for new biomonitoring methods in environmental risk assessment that can incorporate both biodiversity and ecosystem function in a quick and cost-efficient manner. The emerging field of environmental DNA (eDNA) metabarcoding offers such an approach, as it can provide high-throughput, low-cost identification of the presence of multiple species in an area. To test the effectiveness of this new technology in an applied setting, eDNA was collected from three stream locations at The Wilds Conservation Center in southeastern Ohio. The three sites are in a region heavily impacted by acid-mine drainage (AMD) from historical coal-mining operations and represent three distinct scenarios (High AMD, Low AMD, Remediated). eDNA samples were collected monthly (Oct 2019-Feb 2020) via filtration of 1L of stream water. Additionally, leaf litter bags were deployed on the first sampling date and collected monthly to assess differences in decomposition rates and associated macroinvertebrate community structures as determined by metabarcoding. A total of 15 water samples and 12 leaf litter samples were sequenced using 12S primers targeting fish and COI primers targeting macroinvertebrates. The 12S sequencing identified 19 distinct fish species and 17 bycatch species (i.e. birds, mammals). The COI sequencing results were paired with functional trait database, resulting in 33 unique macroinvertebrate genera with associated traits. The leaf litter decomposition rates ($k$) were calculated as -0.0016 day $^{-1}$ (High AMD), -0.0047 day $^{-1}$ (Low AMD), and -0.0053 day $^{-1}$ (Remediated). One fish species, 7 bycatch species, and 6 macroinvertebrate genera were detected at High AMD while 6 fish species, 9 bycatch species, and 14 macroinvertebrate genera were detected at Low AMD. The remediated site had markedly higher detections with 17 fish species, 7 bycatch species, and 26 macroinvertebrate genera. The remediated site was also the only site to have macroinvertebrate genera associated with the 5 primary functional feeding groups including 4 distinct genera of shredders. The results of this study show how routine stream monitoring in mining impacted streams can be accomplished using eDNA. Additionally, the expanded biodiversity information provided by eDNA and integration of other resources such as functional trait databases can allow environmental managers to make more informed decisions about the efficacy of remediation strategies deployed in streams.

### 3.03.P-Tu068 The Efficiency of Washing Techniques To Eliminate External Contamination of Trace Metals in Bat Fur and Bird Feathers.

***Jenna Keute** and Beatrice Hernout, Clarkson University*

Non-invasive proxies, such as fur and feathers, are likely to be increasingly used to assess potential exposure of chemicals including trace metals. The amount of external contamination is usually unknown, and to date, there is no standard method for removing external contamination of trace metals in fur or feathers. About 40% of studies (53 total articles) related to the measurement of trace metals levels in fur or hair of non-human mammals and 24% of studies (92 total articles) in feathers did not state any washing methods or did not wash the samples before analysis.

Firstly, three washing techniques were assessed to remove external contamination of arsenic (As), lead (Pb) and zinc (Zn) in bat fur. We selected the three most frequently used washing methods: *M1*) involving the reagents acetone, detergent and water, *M2*) acetone and water (with repeated steps), *M3*) a simplified method using acetone and water. To test these methods, fur samples of great flying foxes (*Pteropus neohibernicus neohibernicus*, n=30 individuals) from Papua New Guinea preserved over eight decades (AMNH, USA) were used. Levels of metals measured in fur after were significantly lower than in unwashed fur by 90.11%, 94.94%, 91.90% of As, 61.96%, 63.29%, 64.84% of Pb, and 76.35%, 45.58%, 43.76% of Zn, using M1, M2 and M3, respectively. M1 was the most efficient at removing external contamination. Further data regarding the concentrations of metals contained in the aliquots of reagents used during the washing steps will be presented. Regarding potential toxicity, our results show that 33% of the bats contained As levels (> 50 ppm) and 37% of contained Pb levels in hair (> 0.2 ppm) associated with potential adverse health effects.

Secondly, we identified the most prevalent washing techniques in the literature used for feathers: *M1*) acetone and water; *M2*) detergent and water; *M3*) acetone, detergent, and water; and *M4*) acetone, detergent and diluted nitric acid. We used feathers of the great horned owl (*Ardea Herodias*) and the great blue heron (*Bubo virginianus*) to test these methods. Percentages of trace metal removed using M2 are 39.72% for Copper, 10.2% for As, 33.89% Zn, and 6.73% for Mercury. Further statistical analyses and analyses of aliquots are currently underway.

This study shows the importance of washing fur and feather samples prior to trace metals analyses in ecotoxicological and biomonitoring studies.

### 3.03.P-Tu069 The Use of Fish Scale Hormone Concentrations as a Non-Lethal Biomonitoring Tool in Teleost Fishes

***Emily Kennedy** and David Janz, University of Saskatchewan, Canada*

In an effort to monitor changes in stress in wild populations, the use of integumentary structures capable of incorporating steroid hormones over long periods of time has been employed. Traditionally this included hair in mammals and feathers in birds; however, in recent years the use of fish scales for similar purposes is being explored. Today's fish populations are faced with numerous anthropogenic threats to endocrine function including (1) increases in physiological stress, (2) resulting reproductive alterations and (3) endocrine disruption mediated by xenoestrogens and other hormone mimics. To date we have quantified nine steroid hormones within the fish scale in an effort to provide a non-lethal means of assessing the magnitude of these threats. Our first study explored changes in scale cortisol (F) as well as DHEA, an androgen and precursor steroid capable of influencing the conversion of F to it's inactive metabolite cortisone (E) in rainbow trout in response to long-term stress. Increases in F, E and DHEA were observed in the scales of stressed trout when compared to controls. In a second study, trout were injected with either F, progesterone or testosterone to examine the relationship between circulating and scale hormones as well as to allow for the additional assessment of reproductive alterations induced both by stress and endocrine disrupting compounds in future studies. Although these methods are still under development, our results provide promising evidence of the practicality of fish scale hormone concentrations as a non-lethal biomonitoring tool in teleost fish.

## 3.03.V Non-invasive Techniques to Biomonitor Exposure and/or Effects from Anthropogenic Pollutants in Wildlife

### 3.03.V-01 Mercury Chemoscape in the Gulf of Saint-Lawrence: Using Northern Gannets as a Biological Sampling Platform

***Rose Lacombe**[1], Pauline Martigny[2], David Pelletier[3], Magella Guillemette[2], Marc Amyot[4], Raphaël Lavoie[5] and Kyle Elliott[1], (1) McGill University, Canada, (2) University of Quebec, Rimouski, Canada, (3) CÉGEP de Rimouski, Canada, (4) University of Montreal, Canada, (5) Environment and Climate Change Canada*

Contaminants are ubiquitous in marine environments, but they are not evenly distributed. To better understand how an animal's use of its environment may determine its contaminant load, it is important to know where 'hotspots' of contaminants are located. The Gulf of Saint-Lawrence, Québec, Canada, is an economically and ecologically important ecosystem which hosts a high biodiversity of species, but it can be difficult to measure its level of contamination. To map the chemical landscape, or "chemoscape", of the Gulf of Saint-Lawrence, we collected fish regurgitations ($n$ = 54) from northern gannets (*Morus bassanus*) tracked using GPS devices and analyzed total mercury and compound-specific stable nitrogen isotopes in amino acids (AAs) in the fish muscle. Using the $\delta^{15}N$ signatures in AAs, we standardized fish mercury concentrations. Concentrations were assigned to the most recent gannet dive location and we mapped the chemoscape of mercury for the Gulf of Saint-Lawrence. Contrasting mercury profiles were found in the different regions due to differences in food web structure and trophic magnification. Demonstrating where contaminants accumulate more efficiently in the Gulf of Saint-Lawrence is crucial to understanding what risks wildlife are exposed to based on their habitat and feeding ecology.

## 3.04.V PFAS and Related Compounds in Terrestrial and Aquatic Wildlife: Exposure, Uptake, Tissue Distribution, and Toxic Effects

### 3.04.V-01 Omics-Based Assessment of Wild-Caught Freshwater Turtles Exposed to Elevated PFAS Concentrations

***David J. Beale***[1]*, Sandra Nilsson*[2]*, Viviana Gonzalez-Astudillo*[2]*, Christoph Braun*[3]*, Suzanne Vardy*[3] *and Brenda Baddiley*[3]*, (1) CSIRO, Australia, (2) The University of Queensland, Australia, (3) Department of Environment and Science, Queensland Government, Australia*

PFAS are a common contaminant in the environment and identifying constituents, bioaccumulation, and biological impacts of mixtures in wildlife remains a challenge. An omics-based ecosurveillance approach was used to investigate the impacts of PFAS pollution on wild-caught freshwater turtles (*Emydura macquarii macquarii*). Twenty-six turtles (13 females and 13 males) were collected; ten turtles from a PFAS impacted site (downstream from an industrial source of PFAS contamination), ten from a reference site, and six control turtles. The serum PFAS concentration was quantified using an established targeted methodology. The serum PFAS concentrations were ten-fold greater at the impacted site (Σ46 PFAS 1992 ± 620 ng/mL for females and 2927 ± 566 ng/mL for males) relative to the control samples (Σ46 PFAS 77 ± 53 ng/mL for females and 36 ± 42 for males). The reference site turtles were free from any measurable PFAS. The female turtles were necropsied to further investigate the impact and bioaccumulation of PFAS on internal organs (liver, muscle, kidney, fat, heart, brain, and ovaries), the gut microbiome, and maternal offloading into eggs. PFAS offloading was found to be 1.6 and 5.3 times higher in the control and impacted PFAS impacted eggs, respectively, compared to maternal serum values. Biochemical profiles of collected serum and tissues were analyzed using proteomics, lipidomics, and metabolomics-based methodologies. These multi-omic profiles demonstrated a positive correlation in the impacted turtles exposed to elevated PFAS with an enhanced purine metabolism, glycerophosphocholines, and an innate immune response, which suggest an inflammation response, metabolic preservation, and re-routing of central carbon metabolites. Conversely, lipid transport and binding activity were negatively correlated. PFAS impacted eggs were significantly elevated in purine metabolism metabolites. The yolks were significantly depleted in lipids and lipid quality tied to growth and development. The gut microbiome community was also impacted; the ratio of Firmicutes-to-Bacteroidetes indicative of host stress and dysfunction was correlated with metabolic function data. This type of omics-based data has the potential to contribute toward the linkage of adverse outcome pathways for turtle populations exposed to PFAS mixtures. Ecosurveillance and integrated omics have the potential to inform mechanistic toxicological data for risk assessment and regulatory applications.

### 3.04.V-02 Novel Polyfluoroalkyl Betaines Persist in Oxic Soils and Bioaccumulate in Earthworms

***Min Liu***[1]*, Gabriel Munoz*[2]*, Juliana Hermiston*[3]*, Sung Vo Duy*[2]*, Eric Bottos*[3]*, Jonathan Van Hamme*[3]*, Sébastien Sauvé*[2] *and Jinxia Liu*[1]*, (1) McGill University, Canada, (2) University of Montreal, Canada, (3) Thompson Rivers University, Canada*

Contemporary aqueous film-forming foams (AFFFs) contain fluorotelomer betaines (FTBs) with n:3 and n:1:2 (n = 5, 7, 9, 11, 13 and 15) polyfluoroalkyl chains as the major components. They have been frequently detected in AFFF-impacted environments (e.g., soil, surface water, sediment) and biota in recent years, but their environmental fate in terrestrial systems and bioaccumulation potential are largely unexplored. In this study, we will discuss new findings on their biotransformation potential, body burdens in earthworms, and biota soil accumulation factors (BSAFs) in *Eisenia fetida*. 5:3 and 5:1:2 FTB and a commercial AFFF were incubated in four oxic soils with distinct microbial communities. The 120-d experiments showed that these betaines are highly persistent in oxic soil microcosms, as evidenced by the constant levels of the parent betaine(s) concurrent with little or no production of both quantitative and qualitative transformation products. Specifically, no n:3 polyfluoroalkyl acids (n = 2~5) and perfluoroalkyl carboxylic acids (C3~C6 PFCA) were formed from 5:3 FTB, no short-chain hydrogen-substituted polyfluoroalkyl acids (n:2 H-FTCA, n = 1~5) and hydrogen-substituted PFCAs (2H-PFCA, n = 3 ~ 7) were produced from 5:1:2 FTB. In four soils, the commercial AFFF produced trace-level PFCAs at yields of 0.023~0.25 mol%, but these PFCAs were most likely resultant from n:2 fluorotelomers (minor AFFF components) rather than n:3 or n:1:2 FTBs. Furthermore, we determined BSAFs of n:3 or n:1:2 FTBs using laboratory bioaccumulation tests and field observation data. We found that these betaine compounds are less bioaccumulative than perfluoroalkyl acids of the same perfluoroalkyl moiety but remain moderately bioaccumulative. In particular, the earthworms collected from a fire-equipment testing site at a major Canadian airport showed high body burdens of these betaines, along with other AFFF constituents. Similar to other per- and polyfluoroalkyl substances, BSAFs of n:3 and n:1:2 FTBs are related to the perfluorinated chain length. The findings confirm that these fluorotelomer compounds are persistent in soil microcosms and invertebrate species with limited biotransformation or metabolization.

**3.04.V-03 Solutions to the PFAS crisis through use-inspired basic research systems approaches**

***Rebecca C Jordan*** *and Cheryl A. Murphy, Michigan State University*

Widespread contamination of our drinking water and natural and manufactured ecosystems from thousands of PFAS chemicals (~10,000) is a challenging problem that impacts many aspects of our society today. Despite decades of research and thousands of research studies, we still have yet to converge on and implement manageable solutions. The Center for PFAS Research at Michigan State University is predicated on the notion that researchers must engage this PFAS challenge not only within environmental chemistry and toxicology but also more broadly within the life sciences, sociology, and in studies of governance and institutional change. Studies, therefore, about approaches and tools that facilitate learning about, communication on, and remediation of PFAS compounds in such a way that human risk can be mitigated and environmental systems repaired are critical. We used a participatory approach to develop "use-inspired basic" research as a systems approach where researchers and end-users worked together to target research outcomes. Our approach identified our top 10 research questions that lend themselves to solutions-based research outcomes but that also unite members of group to target collaborative research projects and funding sources. These questions are related to identifying, detection and useage of the variety of PFAS, as well as remediation approaches to both natural and manufactured systems, but also suggest research directed on how to change social structures and policy institutions for PFAS solutions. We discuss the main outcomes of our activities surrounding our participatory modeling and the convergence and progress on our guiding research questions.

## 3.04A PFAS and Related Compounds in Terrestrial and Aquatic Wildlife: Exposure, Uptake, Tissue Distribution, and Toxic Effects

**3.04A.T-01 PFAS and related compounds in terrestrial and aquatic wildlife: A brief overview of current understanding of PFAS sources, cycling, and wildlife exposures and effects**

***Cheryl A. Murphy***[1]*, Michael W. Murray*[2]*, Christine M. Custer*[3] *and Natalie K. Karouna-Renier*[3]*, (1) Michigan State University, (2) National Wildlife Federation, United States, (3) U.S. Geological Survey*

Per- and polyfluoroalkyl substances (PFAS) are receiving increasing attention by the scientific, policymaking, industry, and nongovernmental organization communities, as well as the general public. In spite of significant research over the past two decades on multiple aspects of PFAS in the environment, a number of questions remain, including on the extent of PFAS exposure and effects in a diverse range of wildlife species. This overview will review current understanding of PFAS sources and cycling in the environment, as well as approaches to studying exposures and effects in aquatic and terrestrial wildlife in a wide range of ecosystems. Field studies can provide information on uptake of PFAS by individual organism groups (bioaccumulation), as well as, the potential for increasing concentration at higher trophic levels (biomagnification), and biological effects. Because there are an increasing number of species serving as biomonitors of PFAS contamination from around the world, assessments of trends are possible. Laboratory studies are examining uptake, tissue distribution, and elimination, as well as potential effects of PFAS, across a range of model organisms. Research challenges include addressing the thousands of potential PFAS compounds, complex environmental cycling and organism physiological changes, and multiple potential endpoints of concern in wildlife. These challenges underscore the need for widespread collaboration and a broad research focus to address key aspects of PFAS ecotoxicology engaging with multiple disciplines. This session will provide a forum for researchers to present information addressing a number of these issues, and will inform key questions to consider in future PFAS-related studies.

**3.04A.T-02 Target and non-target analysis of per- and polyfluoroalkyl substances (PFAS) in surface water following an industrial fire of unknown chemical stockpiles**

***Jaye Marchiandi***[1], *Bradley Clarke*[1], *Drew Szabo*[1], *Mark Green*[1], *Sahil Rana*[1] *and Sonia Dagnino*[2], *(1) The University of Melbourne, Australia, (2) Imperial College London, United Kingdom*

An industrial warehouse illegally storing a large quantity of unknown chemical and industrial waste ignited in an urban area in Melbourne, Australia. The multiday fire required firefighters to use large amounts of fluorine-free foam that carried contaminated firewater runoff into an adjacent freshwater creek. In this study, the presence of per- and polyfluoroalkyl substances (PFAS) was investigated using targeted and non-targeted analysis on triplicate surface water samples ($n = 45$ and 15, respectively) from five locations over three sampling campaigns (2018 to 2020). Out of the 42 targeted PFASs, perfluorocarboxylates (PFCAs: C4-C14), perfluoroalkane sulfonates (PFSAs: C4-C10), and perfluoroalkyl acid precursors (e.g. 6:2 fluorotelomer sulfonate (6:2 FTSA)) were ubiquitously detected in surface waters (concentration ranges: <0.7-3000 ng/L). Emerging PFASs including a cyclic perfluoroalkanesulfonate (PFECHS) and a C4 perfluoroalkane sulfonamide (FBSA) were also repeatedly present in surface water (concentration ranges <0.3-77 ng/L). A significant difference in ΣPFAS concentration was observed at the point-source (mean 5500 ng/L; 95% CI: 4800, 6300) relative to upstream sites (mean 100 ng/L; 95% CI: 90, 110; $p \leq 0.001$). The point-source ΣPFAS concentration decreased from $5500 \pm 1200$ ng/L to $960 \pm 42$ ng/L (-83%) after two months and to $430 \pm 15$ ng/L (-98%) two years later. Non-targeted analysis of samples collected during firefighting identified several novel and emerging fluorotelomer-based fluorosurfactants, including fluorotelomer sulfonamido betaines (6:2, 8:2, 10:2, 12:2 FTAB), fluorotelomer thioether amido sulfonic acid (6:2 FTSAS), and fluorotelomer sulfonyl amido sulfonic acid (6:2 FTSAS-So). Notably, we tentatively identified ethyl 2-ethenyl-2-fluoro-1-(trifluoromethyl) cyclopropane-1-carboxylate, a novel short-chain PFCA, for the first time. Analysis of several Class B certified fluorine-free foam formulations permitted for use in Australia revealed no detectable PFAS. Homologue profiles of PFAS detected in surface water are consistent with environments impacted by fluorinated aqueous film-forming foams. Therefore, these results provide strong evidence that firewater runoff of stockpiled fluorinated firefighting waste was the dominate source of detectable PFAS to the surrounding environment. Our study emphasizes that the stockpiling of PFAS waste may represent an emerging global problem in regard to long-term environmental PFAS contamination.

**3.04A.T-03 Impact of Sediment Organic Carbon on Per- and Polyfluoroalkyl Substances Bioaccumulation in Freshwater Macroinvertebrates**

***Xiaoyan Yun***[1], *Asa J. Lewis*[2], *Christopher Sales*[2], *Daniel E. Spooner*[3], *Marine J. Kurz*[2,4], *Rominder Suri*[1] *and Erica R. McKenzie*[1], *(1) Temple University, (2) Drexel University, (3) Lock Haven University, (4) Oak Ridge National Laboratory*

Per- and Polyfluoroalkyl Substances (PFAS) have caused globally concern due to persistence and toxicity of some compounds, particularly perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) which exhibit a tendency to bioaccumulate. Sediment is an important compartment of aquatic ecosystem; while the sediment fraction of organic carbon impacts PFAS partitioning between aqueous and solid phases, little is known about the influence of sediment organic carbon content on PFAS bioaccumulation in benthic organisms. In this study, a bioaccumulation test was performed with three treatments – 2%, 5% and 8% sediment organic carbon – to study the sediment organic carbon effect on 14 PFAS (8 perfluoroalkyl carboxylic acids, 3 perfluoroalkyl sulfonic acids and 3 fluorotelomer sulfonic acids) bioaccumulation in benthic invertebrates. Three freshwater benthic macroinvertebrates, including worms (*lumbriculus variegatus*), mussels (*elliptio complanata*) and snails (*physella acuta*), were exposed to PFAS spiked synthetic sediment equilibrated with a synthetic surface water for 28 days. Increasing sediment organic carbon content decrease the bioavailability of PFAS to benthic invertebrates; for worms, mean PFOS concentration is 322, 277 and 79 ng/g wet weight in 2%, 5% and 8% sediment organic carbon, respectively. For compounds with the same perfluoroalkyl chain length, the bioaccumulation factors and biota-sediment accumulation factors in general following a trend of perfluoroalkyl carboxylic acids < perfluoroalkyl sulfonic acids < fluorotelomer sulfonic acids, indicating the less evaluated fluorotelomer sulfonic acids warrant greater attention. Worms exhibited substantially greater bioaccumulation compared to mussels and snails, likely related to different exposure pathways and metabolism capacities. This suggests that worms can be used as a potential biomonitoring species. Understanding the impact of sediment organic carbon on PFAS bioaccumulation in benthic organisms is key to understanding PFAS incorporation into the aquatic food web and associated potential ecological risks to higher trophic level wildlife such as fish and water birds.

**3.04A.T-04 Assessing potential perfluoroalkyl substances (PFAS) trophic transfer to crickets (Acheta domesticus)**

***Kaylin McDermett,*** *Todd Anderson, William Jackson and Jennifer Guelfo, Texas Tech University*

Although many studies have assessed the bioaccumulation of perfluoroalkyl substances (PFAS) in plant tissues, to date, there has been minimal research on the bioaccumulation of PFAS in soil invertebrates that results from consuming PFAS-contaminated media. This study focused on two different consumption pathways in a population of crickets: individuals consuming PFAS-contaminated alfalfa (*Medicago sativa)*, and individuals consuming PFAS-spiked drinking water. Alfalfa was grown in a greenhouse and irrigated with PFAS-spiked water (~1 ppm) containing seven unique PFAS. The alfalfa was then harvested and fed to crickets. Another population of crickets was supplied with PFAS-spiked drinking water at similar concentrations for direct consumption. Alfalfa tissue accumulation of PFAS and subsequent consumption by the crickets resulted in overall similar cricket tissue concentrations to the crickets who consumed PFAS-spiked water directly. This indicates that source concentration (water) may play a key role in assessing bioaccumulation of PFAS up the food chain. Additional critical information about PFAS chain-length on bioaccumulation to the crickets was also explored, and bioconcentration factors (BCFs) for the seven PFAS of interest were calculated. To our knowledge, this is the first study which has assessed not only the direct potential of PFAS trophic transfer from contaminated vegetation, but also highlights the similarities in bioaccumulation, regardless of ingestion pathway or plant accumulation. As PFAS bioaccumulation and biomagnification in food webs are topics that remain in question, this work aimed to provide more understanding of how PFAS enters the food web through soil invertebrates.

**3.04A.T-05 Characterizing and Comparing Bioaccumulation of Complex PFAS Mixtures in Aquatic Food Webs Affected by Different PFAS-sources**

***Robert Wesley Flynn***[1]*, David Lee Haskins*[2]*, Youn Jeong Choi*[2]*, Matthew T. Hamilton*[2]*, Melissa E. Lech*[2]*, Linda S. Lee*[2]*, Marisol S. Sepulveda*[2] *and Jason T. Hoverman*[2]*, (1) U.S. Geological Survey, (2) Purdue University*

Per- and polyfluoroalkyl substances (PFAS) are contaminants of emerging concern that are commonly detected in surface waters globally. Increased research efforts in recent decades have provided insight into the spatial extent of PFAS contamination and potential health effects for humans and wildlife, but substantial knowledge gaps remain that can impede data-driven risk assessment. In the environment, PFAS most often occur as complex mixtures, whose composition is determined in part by local sources. However, how different PFAS sources influence environmental mixtures and how the components of these mixtures move through food webs is not well characterized. We examined PFAS accumulation in the producers and consumers (invertebrates and vertebrates) of two aquatic food webs, each impacted by a distinct PFAS source; one associated with use of aqueous film forming foams and another associated with tannery waste. We examine accumulation profiles within and between these food webs to provide insight into how source influences PFAS accumulation profiles, the role of trophic level on within and among species variation in accumulation, and the associated implications for wildlife and human health. Our data highlight the complex nature of PFAS accumulation and exposure in the environment; indicating the importance of the diet on exposure risk in aquatic taxa and that perfluorooctane sulfonate (PFOS) may not always account for ≥ 50% of PFAS body burdens.

**3.04A.T-06 Assessing Exposure to PFAS from Different Sources Using a Model Species: The Tree Swallow**

***Christine M. Custer****, Paul M. Dummer and Natalie K. Karouna-Renier, U.S. Geological Survey*

While exposure of wildlife to per- and polyfluoroalkyl substances (PFAS) can come from many sources, one prominent source are facilities that use aqueous film-forming firefighting foams (AFFF), both operationally and for training. Other sources include manufacturing facilities and industrial plants that use PFAS-containing products. The objective of this study is to use a model avian species, the tree swallow (*Tachycineta bicolor*), to assess exposure to and quantify effects, if any, of PFAS at Department of Defense (DoD) installations in the Mid-Atlantic region, USA compared to reference locations, and compared to locations with other PFAS sources. Tree swallow nest boxes were sampled in 2020 and 2021 at selected military installations (AFFF source), two reference locations at the Patuxent Research Refuge, Laurel, MD, and at two sites near the Twin Cities, MN which have other PFAS sources. Tree swallow nesting was monitored weekly, and egg, nestling, and diet samples were collected at the appropriate times in the nesting cycle. Samples were analyzed for 33 PFAS, as well as, for biomarker responses. Two levels of effects were assessed relative to PFAS and other contaminant exposures. At the population level, reproductive effects were measured as percent hatching and daily probability of nest success. Biomarker responses, which are indicative of whether exposure is high enough to elicit even a physiological response in individual birds, was compared to normal responses in reference swallows and relative to PFAS exposure. There were different levels of PFAS exposure across study sites, and also differences in the patterns of specific PFAS depending on the source. These differences will allow for a robust assessment of which PFAS are contributing to effects, if any effects are documented.

**3.04A.T-07 Spatiotemporal trends of PFAS in Herring Gulls in the Great Lakes**

***Matthew Shuwal*[1]*, Chunjie Xia*[1]*, Kevin A. Romanak*[1]*, Keith Grasman*[2]*, Marta Venier*[1] *and Mandy Annis*[3]*, (1) Indiana University, Bloomington, (2) Calvin University, (3) U.S. Fish and Wildlife Service*

Perfluroalkyl substances (PFAS) in the Great Lakes have been a rising concern due to the strong bioaccumulation potential and their associated complex ecotoxicities. In this study, we evaluated 75 PFAS in herring gull (*Larus argentatus*) serum. Sera (n=200) were collected from Saginaw Bay Confined Disposal Facility, Saginaw Bay Little Charity Island, Bellow Island, Detroit-Edison Power Plant, and Pipe Island in the Great Lakes basin between 2010 and 2021. Target PFAS included perflurocarboxylic acids (PFCAs), perflurosulfonic acids (PFSAs), flurotelomere sulfonic acids (FTS), fluoroalkylphosphinic acids (PFPi), Ethers, Perfluoroalkane sulfonamides (FASAs), perfluorooctane sulfonamido acetic acids (FOSAAs), flurotelomere unsaturated carboxylic acids (FTUCAs), and polyfluoroalkyl phosphate esters (PAPs). The most abundant PFAS detected is perflurooctane sulfonic acid (PFOS) with concentrations ranging from 20 - 300 ng/g ww. Perflurocarboxylic acids with chain length between 9 and 13 were several orders of magnitude more concentrated in samples than shorter chain PFCAs. Gulls sampled at Pipe Island had the highest levels of PFCAs for the 5 locations included in the study. The main objectives of this study were to measure and compare legacy and emerging PFAS in herring gulls from the Great Lakes and determine spatial and temporal patterns in over a decade worth of data. As far as the authors are aware, this is the first study on PFAS levels in herring gull serum for the great lakes ecosystem.

**3.04A.T-08 Investigation of Per- and Polyfluoroalkyl Substances (PFAS) in Surface Water, Sediment, and Aquatic Vegetation in Florida**

***Emily Griffin*** *and John A. Bowden, University of Florida*

Per- and polyfluoroalkyl substances (PFAS) constitute a class of highly stable and extensively manufactured anthropogenic chemicals that have been linked to a variety of adverse health effects in humans and wildlife. These compounds are ubiquitously distributed in the environment and have been measured in aquatic systems globally. Florida manatees (*Trichechus manatus*) are particularly vulnerable to exposure as they inhabit shallow, coastal waters, often near high industrial activity, such as airports, air force bases, and wastewater treatment facilities. However, there is a fundamental gap in the understanding of how environmental stressors, such as PFAS, integrate into aquatic herbivores and what the related health effects may be. Our group has measured PFAS in manatee blood throughout Florida and Puerto Rico, yet, the exposure route of PFAS for herbivores is unknown, in addition to understanding how these chemicals partition into different environmental matrices. The distribution of PFAS in the environment depends on the physiochemical properties of the individual PFAS and the hydrological characteristics of the aquatic system. The objective of this study was to investigate the extent of PFAS contamination in Florida manatee habitats in an effort to identify potential routes of PFAS exposure. Surface water (n=154), sediment (156), and aquatic vegetation (n=175) were collected at eleven sites throughout Florida (with an average of 10 locations sampled at each site). Surface water was extracted via solid phase extraction (SPE) and sediment and vegetation employed a liquid-liquid rotation extraction. All samples were analyzed by ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) and monitored for over 92 PFAS. For the first time, PFAS have been recorded in aquatic vegetation and compared to concentrations identified in matched surface water and sediment across eleven aquatic environments. Data from this study will provide novel insights to how PFAS integrate into various matrices within coastal environments. Characterizing PFAS in the manatee environment enhances understanding of exposure thereby contributing to the ongoing conservation efforts of this threatened and protected species.

**3.04A.P PFAS and related compounds in terrestrial and aquatic wildlife: Exposure, uptake, tissue distribution, and toxic effects**

**3.04.P-We087 Per- and Polyfluoroalkyl Substance Uptake into Shellfish in Cape Cod Massachusetts**

***Christopher J. McCarthy***[1]*, Barrie Selcoe*[1]*, Nigel Tindall*[1] *and Rose Forbes*[2]*, (1) Jacobs Engineering Group Inc., (2) Air Force Civil Engineer Center*

Shellfish were collected on the coast of Cape Cod Massachusetts for the analysis of Per- and polyfluoroalkyl substances (PFAS). PFAS were initially detected in groundwater, porewater, and surface water samples at and near Hen Cove and Red Brook Harbor as part of an investigation of two historical aqueous film forming foam (AFFF) application sites situated upgradient of the waterbodies. The AFFF applications resulted in PFAS contaminated groundwater migrating to the two coastal inlets where recreational shell fishing is allowed. Quahogs (*Mercenaria mercenaria*) and oysters (*Crassostrea virginica*) were identified by the local Shellfish Constables as the most commonly collected shellfish in the receiving waters that are harvested for human consumption. These species were also the most likely to have sufficient population densities with body mass within limits of recreational shellfish permits and meeting analytical requirements. Quahogs and oysters were collected from a total of four study areas within Hen Cove and Red Brook Harbor and two reference area locations. Samples were collected in coordination with the Town of Bourne and Town of Falmouth Departments of Natural Resources. Three samples were collected from each study area for each target species as a composite of tissue from ten individual organisms. Sediment, surface water, and pore water samples representing the exposure areas to these organisms were collected concurrently. Tissue samples were analyzed by liquid chromatography tandem mass spectrometry using modified EPA Method 537.1. Abiotic media samples were not analyzed for PFAS but were archived for potential future analysis. Concentrations of Perfluorooctanoic Acid (PFOA), Perfluorooctane Sulfonic Acid (PFOS) and Perfluorobutane Sulfonic Acid

(PFBS) in shellfish tissue will be compared to the site-specific shellfish screening levels protective of consumption by humans. PFOA, PFOS and PFBS tissue data from Hen Cove and Red Brook Harbor will also be compared to results from reference areas. Finally, the tissue data will be used to qualitatively assess ecological risk. Results will be used to support the risk assessments for the contaminated site investigations of the two AFFF application sites. Pending shellfish PFAS sample results comparisons to screening levels, sediment, surface water, and pore water samples may be analyzed for PFAS and be assessed to better understand PFAS uptake by the shellfish.

**3.04.P-We088 Bioaccumulation of Per- and Polyfluoroalkyl Substances in Delaware Bay Ecosystem**

***Shannon Jones** and Miling Li, University of Delaware*

Per- and polyfluoroalkyl substances (PFAS) are a large group of anthropogenic chemicals which are used in the manufacturing of various commercial products. Their presence has been linked to multiple adverse health effects in both humans and wildlife. Nearshore ecosystems are particularly vulnerable to PFAS, due to the water solubility of PFAS and high input of potentially-contaminated water from anthropogenic sources. Prior studies have shown variation in bioaccumulation of PFAS by compound, by organism, and between ecosystems. Here, we use the Delaware Bay as a study site to elucidate the factors driving differential bioaccumulation of PFAS in organisms of nearshore estuaries, including primarily ecosystem characteristics (e.g., salinity, proximity to PFAS point-sources, dissolved organic carbon) and physicochemical properties of the given PFAS compounds (e.g., functional group, carbon-fluorine chain length). We collect high site-fidelity organisms across trophic levels throughout the Delaware Bay for PFAS analysis. Preliminary results show higher accumulation of PFAS in finfish than in shellfish. While concentration and composition differ between species, PFAS precursors such as Perfluorooctanesulfonamide (FOSA) make up a much larger proportion of the total PFAS in shellfish than in finfish. We will compare the trophic magnification factor and biomagnification factor of each PFAS compound between the separate food webs of the upper and lower Delaware Bay, to associate the differences in biomagnification with ecosystem and PFAS compound-specific characteristics. The outcome of this study will shed light on the mechanisms of PFAS bioaccumulation throughout the coastal food web.

**3.04.P-We090 Per-/polyfluoroalkyl substances (PFAS) in reptiles from Clark's Marsh Wildlife Area**

***David Lee Haskins**[1,2], Robert Wesley Flynn[1], Matthew T. Hamilton[1], Melissa E. Lech[1], Youn Jeong Choi[1], Linda S. Lee[1], Marisol S. Sepulveda[1] and Jason T. Hoverman[1], (1) Purdue University, (2) U.S. Geological Survey*

Per- and polyfluoroalkyl substances (PFAS) are compounds of significant global concern. These chemicals have been used in a variety of consumer products including cosmetics, food packaging, non-stick cookware, biosolids, and aqueous film forming foams (AFFFs). PFAS-containing AFFFs have been used extensively at Department of Defense Fire/Crash/Training sites since the 1970s. The widespread usage of PFAS combined with their high resistance to degradation has contributed to PFAS contamination of soils, water, and sediment at these sites and surrounding areas. Clark's Marsh Wildlife Area has emerged as an area of exceptional concern due to high levels of PFAS contamination resulting from historical AFFF use at the adjacent Wurtsmith Air Force Base. Although PFAS concentrations reported in wildlife at Clark's Marsh are known to be remarkably high, PFAS accumulation in reptiles at the site is poorly understood. Internationally, there is a paucity of information related to PFAS accumulation and distribution in reptiles. Reptiles are important components of natural ecosystems and can serve as effective bioindicators of local contamination. We report preliminary results of PFAS concentrations from three tissues (liver, muscle, and plasma) of four reptile species: painted turtles (*Chrysemys picta*), common snapping turtles (*Chelydra serpentina*), Northern watersnakes (*Nerodia sipedon*), and eastern garter snakes (*Thamnophis sirtalis*). Average ∑ PFAS (± SE) in reptiles was highest in *C. serpentina* (8089 ± 2688 ng/g ww, n = 5), followed by *N. sipedon* (5663 ± 2688, n = 4), *C. picta* (1603 ± 218, n = 12), and *T. sirtalis* (896, n = 1). We will also provide further descriptions of 23 specific PFAS in multiple tissue types from all sampled individuals. Our dataset represents an opportunity to provide critical PFAS bioaccumulation data for use in risk assessments, as well as a foundation for future studies focused on the potential effects of PFAS on reptiles.

### 3.04.P-We091 Occurrence and Bioaccumulation Patterns of Per- and Polyfluoroalkyl Substances (PFAS) In the Marine Environment

***Bushra Khan**, Robert M. Burgess and Mark Cantwell, U.S. Environmental Protection Agency*

Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic organofluorine compounds used in many commercial applications, household products, and industrial processes since the 1940s. The concern around the persistence, bioaccumulation, and toxicity of these contaminants continues to rise. The objective of this summary is to review the scientific literature to compare patterns of PFAS accumulation in marine organisms and identify compounds of greatest potential concern. To accomplish this, we synthesized data of PFAS occurrence and accumulation in seawater, sediments, plankton, invertebrates, fish, reptiles, birds, and mammals from marine habitats. The summarized data presented here include PFAS concentrations across tissue types and biological compartments from studies published between the years 2000 and 2020. Long-chain perfluoroalkyl carboxylic acids, particularly perfluoroundecanoic acid (PFUnA), were detected at high concentrations across different taxa as well as across temporal studies indicating their persistence and bioaccumulative potential. Perfluorooctanesulfonic acid (PFOS) was also elevated in several tissue types across taxa. Further, precursors and replacement PFAS were detected in several marine organisms. Taxonomic and tissue-specific differences in accumulation were noted, e.g., PFAS were observed to be elevated in protein-rich tissues. Differences between bony and cartilaginous fishes were also noted. Accumulation patterns indicate higher levels of PFAS in air-breathing organisms (seabirds and marine mammals) than in those that respire in water, suggesting differences in elimination kinetics. Additionally, this review also highlights research needs and challenges related to PFAS biomonitoring including (i) effects of environmental and biological variables (e.g., dietary, metabolic and physiological), (ii) evaluation of protein binding sites and affinities, and (iii) biotransformation of precursors. Identification of these accumulation trends across habitats and taxa can be applied towards the design of biomonitoring efforts, determination of high-risk marine taxa, derivation of criteria development for the most concerning PFAS, and performance of risk assessments.

### 3.04.P-We092 Per- and Polyfluoroalkyl Substances (PFAS) in Archived Marine Biota from Rhode Island, USA

***Melanie L Hedgespeth**[1], David Taylor[2], Maya Morales-McDevitt[3], Sawyer Balint[3], Morgan Schwartz[1] and Mark Cantwell[1], (1) U.S. Environmental Protection Agency, (2) Roger Williams University, (3) Oak Ridge Institute for Science and Education (ORISE) participant at U.S. Environmental Protection Agency*

Per- and polyfluoroalkyl substances (PFAS) are highly persistent, fluorinated compounds that are frequently detected in environmental and biological samples. Many legacy PFAS accumulate in organism tissues, in some instances resulting in biomagnification across trophic levels. Further, some PFAS have been linked to adverse health impacts in humans and other organisms. Our study conducted a retrospective analysis of 24 PFAS in archived marine biota samples, for which we detected PFAS from sub-classes including PFCAs (per- and polyfluoroalkyl carboxylic acids), PFSAs (per- and polyfluoroalkyl sulfonic acids), and precursor compounds. Samples were originally collected from 2006-2014 within an urban estuary (Narragansett Bay, RI) and in adjoining offshore waters (Rhode Island/Block Island Sounds). We examined trends in PFAS concentrations related to differences among species and trophic positions/feeding guilds, as well as spatial and temporal patterns related to collection. Results indicate marked inter- and intraspecies variation in overall PFAS concentrations and composition, with $\sum^{24}$PFAS detected up to ~20 ng/g wet weight. PFOS, PFTrDA, or FOSA were detected at the highest concentrations in most species. Organisms collected inshore and from high trophic levels tended to contain the highest PFAS concentrations detected in the study, e.g. striped bass, though some crustaceans also contained relatively large PFAS concentrations. Further, we used these data to gauge the potential for human exposure due to the consumption of commercially and recreationally important species.

### 3.04.P-We093 PFAS in Large Predator Salmonids from Lake Michigan (USA)

***Daniele Miranda***[1], *Alison M. Zachritz*[1], *Heather D. Whitehead*[1], *Shannon R. Cressman*[2], *Graham F. Peaslee*[1] *and Gary A. Lamberti*[1], *(1) University of Notre Dame, (2) U.S. Fish and Wildlife Service*

Per- and polyfluoroalkyl substances (PFAS) can pose serious long-term threats to aquatic ecosystems due to their high persistence, rapid spread in water bodies, and potential for bioaccumulation and biomagnification. PFAS have been linked to hepatotoxicity, developmental alterations, immunotoxicity, and adverse hormonal effects in biota. The main goals of this study were to evaluate whether PFAS have accumulated in Lake Michigan salmonids and if a geographic pattern of PFAS can be detected. PFAS were evaluated in males and females of four salmonid species (i.e., adult lake trout, steelhead trout, coho salmon, and Chinook salmon over 600 mm in length) collected in different quadrants of Lake Michigan (NW, NE, SW, and SE). Muscle tissue samples from 31 salmonids were collected in each of the different quadrants. PFAS concentrations in fish tissue were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). PFAS were observed in all examined salmonid species, with $\sum_{21}$PFAS accumulation being independent of sex ($p>0.05$). Thirteen of the 21 PFAS analytes were found in total average concentrations of 6.5 ng $g^{-1}$ wet weight (ww; range, 0.04–24 ng $g^{-1}$ ww). PFAS concentrations in Lake Michigan salmonids were lower when compared to previous studies that analyzed salmonids from other Great Lakes. PFAS composition was similar in all fish, suggesting similar sources or uptake dynamics. Perfluorononanoic acid (PFNA) was the most frequently detected compound (mean, 0.20 ng $g^{-1}$ ww; range, 0.04–0.74 ng $g^{-1}$ ww), while perfluorooctane sulfonic acid (PFOS) was present in 80% of fish samples at concentrations ranging from 0.87 to 22 ng $g^{-1}$ ww (mean, 7.0 ng $g^{-1}$ ww) and was the dominant PFAS in all fish in terms of concentration. Our results confirm the dominance of PFOS in salmonids from the Great Lakes, even 20 years after its phase-out. Chinook salmon showed higher concentrations of PFAS when compared to coho ($p=0.047$), but had similar concentrations as other salmonids. PFAS did not differ among Lake Michigan quadrants ($p>0.05$), although NW Lake Michigan displayed a trend towards higher concentrations. Overall, our results suggest that PFAS continue to cycle in the Lake Michigan ecosystem and to accumulate in important sportfish at high trophic levels.

### 3.04.P-We094 PFAS Bioaccumulation and Trophic Transfer in Linked Stream and Riparian Food

***Kaitlyn Campbell***[1], *Alison Baranovic*[1], *Ashley Helton*[1], *Anthony Provatas*[1], *David Walters*[2] *and Jessica Brandt*[1], *(1) University of Connecticut, (2) U.S. Geological Survey*

Per- and polyfluoroalkyl substances (PFAS) are a group of anthropogenic substances that are known for their strong fluorine-carbon bonds, resistance to biological degradation, and hydrophobic and lipophilic properties. Due to these unique characteristics, PFAS are used in a wide variety of products and have warranted concern due to their persistence, ability to bioaccumulate, and potential toxicity. PFAS are commonly detected in aquatic ecosystems, and aquatic emergent insects can serve as vectors of waterborne contaminants, such as PFAS, to the adjacent riparian food web. Despite the recent heightened focus on PFAS contamination, few studies have investigated PFAS trophodynamics in linked stream and riparian food webs. The present study focused on PFAS concentration, composition, and trophic transfer in stream and riparian food webs in the Farmington River watershed, Connecticut, USA. Surface water, seston, detritus, biofilm, sediment, aquatic insect larvae, emergent aquatic insects, and riparian spiders were collected in June 2022 and will be analyzed for 28 PFAS. Stable isotope analysis of carbon and nitrogen will also be performed to determine trophic position and to better understand the trophic transfer of PFAS. Based on studies conducted in similar systems, we hypothesize that aquatic insect larvae and emergent insects will have the highest PFAS concentrations, followed by riparian spiders that consumed aquatic prey. Additionally, we anticipate higher detections of short chain PFAS in water samples and long chain PFAS in sediment and biofilm based on chemical persistence and mobility. This study will provide valuable information about PFAS bioaccumulation patterns and trophic transfer in stream food webs as well as quantify aquatic emergent insect flux and PFAS levels transferred to terrestrial predators.

**3.04.P-We095 Assessment of Biomagnification Potential for Per/polyfluoroalkyl Substances in Terrestrial Food-webs Using Mammalian and Amphibian Models.**

***Roman Kuperman**[1], Linnzi K. Wright[1], Michael Simini[1], Ruth W. Moretz[1], Andrew East[2], Michael Quinn[2], Guilherme R. Lotufo[3], Robert Boyd[4], Jennifer Guelfo[5], Zhao Yang[5] and Emily E. Reinders[6], (1) U.S. Army DEVCOM Chemical Biological Center, (2) U.S. Army Public Health Center, (3) U. S. Army Corps of Engineers, (4) U.S. Army Engineer Research and Development Center, (5 )Texas Tech University, (6) Excet Inc.*

Per- and polyfluoroalkyl substances (PFAS) are persistent in the environment, which can lead to their bioaccumulation and biomagnification in terrestrial systems. The main objective of the ongoing research is to determine biomagnification of PFAS compounds in terrestrial food-webs from lower trophic level organisms to higher trophic level organisms using environmentally relevant mixtures. In Phase 1 of the project, we investigated the toxicokinetics of eighteen PFAS compounds in soil invertebrates (earthworms) and determined the rates of uptake, elimination, and bioaccumulation factors (BAF). We also quantified the uptake of these PFAS compounds in terrestrial plants (kale and Timothy grass) from soil and generated bioconcentration factors (BCF). Based on the results of the Phase I investigations, we selected PFAS compounds with BAF/BCF values of two or greater for the Phase II studies. These studies are determining biomagnification factors for the chemical transfer of individual PFAS compounds from (1) plants grown in PFAS amended soil (kale) or purchased and amended via atomizer with PFAS compounds (Timothy grass hay) to mammals (Dutch belted rabbits); and (2) from earthworms exposed in PFAS amended soil to amphibians (American toads), each exposed to PFAS through their daily diet. Ecologically relevant exposure data developed in this research will fill several knowledge gaps regarding biomagnification potential for PFAS compounds released into the terrestrial environment.

**3.04.P-We096 Biomagnification Potentials for a Suite of PFAS Compounds in a Soil-Plant-Mammal Model**

***Michael Simini**[1], Linnzi K. Wright[1], Ruth W. Moretz[1], Roman Kuperman[1], Jennifer Guelfo[2], Zhao Yang[2], Emily E. Reinders[3] and Guilherme R. Lotufo[4], (1 )U.S. Army DEVCOM Chemical Biological Center, (2) Texas Tech University, (3) Excet Inc., (4) U.S. Army Corps of Engineers*

Per/polyfluoroalkyl substances (PFASs) have been linked to accumulation in soil, terrestrial plants and mammals in previous studies with the potential for biomagnification in terrestrial food webs. We investigated the uptake of a suite of PFAS compounds in Timothy grass (*Phleum pratense*) and kale (*Brassica oleracea*) from soil to estimate the biomagnification potential in a soil-plant-rabbit food web. Plants were grown in a modified Organisation for Economic Co-operation and Development (OECD) constructed soil amended with 0.01 or 0.1 mg/kg each of 18 PFAS compounds to generate bioconcentration factors (BCF). The shoot BCFs increased as the C chain length decreased except for Perfluorobutanoic acid (PFBA) and were greater for kale than for Timothy grass. PFBA had the greatest BCF in plant shoots. Root BCFs generally increased with C chain length except for PFBA. PFAS compounds with shoot BCF values ≥2 were selected for the soil-plant-rabbit biomagnification study. For the biomagnification study, kale plants were grown in an environmentally controlled greenhouse in a modified OECD constructed soil amended with 0.01 mg/kg each of 18 PFAS compounds for 56 days. Kale leaves were harvested from mature plants. Certified contaminant-screened Timothy hay was purchased and amended via atomizer with PFAS compounds at concentrations achieved in the BCF studies. Plant material was fed to Dutch-belted rabbits weekly for a 28-day uptake phase and 28-day elimination phase. Accumulation of the 11 compounds in soil, plants and rabbits were analytically determined. Ecologically relevant data developed in this research will fill several knowledge gaps regarding biomagnification potential for PFAS compounds released into the terrestrial environment.

**3.04.P-We097 Assessment of per- and polyfluoroalkyl substances in two species of captive delphinids from United States**

***Leila S. Lemos**[1], Estela Manfrin da Silva[1], Karen J. Steinman[2], Todd R. Robeck[2] and Natalia Soares Quinete[1],*

*(1) Florida International University, (2) SeaWorld Parks and Entertainment*
Delphinids serve as important sentinel species for the health of the environment they live in. They are long-lived top predators potentially at risk from contaminants through the processes of bioaccumulation and biomagnification in the food chain. Among all man-made hazardous substances that may threaten these individuals is a group of chemicals of growing concern that is composed of per- and polyfluoroalkyl substances (PFAS), which are widely used in commercial and consumer products, including food packaging, cosmetics, and personal care products. Thus, the current study investigated the potential contamination of 30 PFAS during different life stages in 28 different individuals of two species of captive delphinids (*Tursiops truncatus* [n = 41], and *Orcinus orca* [n = 44]) located across three SeaWorld Parks in Orlando, FL, San Antonio, TX and San Diego, CA, USA. Blood samples were collected from 1994 to 2020 from each individual's central fluke vein using a 19-gauge needle and syringe into vacutainers containing activated thrombin. Serum was then separated from the whole blood by centrifugation and stored at -80° C. Information on sex, age, maturity, and pregnancy and parturition events were also considered to evaluate PFAS variability among the different demographic profiles as well as investigate potential PFAS transference from mother to calf. For the PFAS extraction, internal standards and methanol were added to each of the samples and vortexed for two minutes. Then, extracts underwent evaporation, cleanup, and filtering processes before being analyzed by liquid chromatography-tandem mass spectrometry. Preliminary results indicate that all PFAS compounds were detected in at least one of the analyzed individuals. Variations in contaminant concentrations across both species, time, location, and demographic profiles as well as maternal transference will be investigated. Correlations between all these variables will also be investigated. Continued monitoring of PFAS concentrations as well as other emerging contaminants in bioindicator species like delphinids is essential to better understand temporal, demographic, and reproductive phase variation as well as potential transference of these contaminants from mother to calf. The methods applied in this study may be extrapolated to populations of wild animals, including endangered species, where the findings of such a study could influence decision-making related to environmental monitoring and management.

**3.04.P-We098 The Impact of Criteria Weighting on a Relative Risk Screening Assessment Framework for Per- And Polyfluoroalkyl Substances (PFAS)**

***Dana McCue**, Ceil Mancini and Gary R Long, EHS Support LLC*

In recent years, the United States Environmental Protection Agency (USEPA) has made several regulatory advancements regarding per- and polyfluoroalkyl substances (PFAS), including developments related to reporting obligations, hazardous substance/air pollutant designation, testing/sampling requests, and restrictions in using PFAS in commerce. However, with most of the over 12,000 PFAS currently identified by USEPA lacking toxicological data, the regulated community faces challenges in how to assess risk to human health and the environment, and how to effectively communicate the risk assessment findings to stakeholders.

A tiered screening assessment framework has been developed and was shared at the SETAC Annual Meeting in 2021. That assessment framework shows the benefit of leveraging approaches currently used for the assessment of industrial chemicals in the United States and internationally. The framework also incorporates approaches used in traditional human health and ecological risk assessments conducted under USEPA's Superfund Program paradigm. The framework categorizes or assigns PFAS into low, medium and high tiers based on their relative risk. Specifically, this categorization approach considers the following elements: human and ecological toxicity data (adverse outcomes, potency, serum elimination half-lives); environmental occurrence, including the potential for biodegradation or transformation; mobility; bioaccumulation/biomagnification; and, site-specific information (source, data set, receptors, pathways). As with other approaches, an uncertainty analysis is conducted with the impact of the uncertainty incorporated into the assessment of relative risks.

The initial model runs did not include criteria weighting. A modified Delphi Group exercise was undertaken to weight the criteria based on inputs provided by toxicologists, ecologists, remediation engineers, risk managers and other decision makers. Incorporation of the weighted criteria has resulted in the classification of PFAS compounds into tiers which more accurately represent relative toxicity. The model is a useful data analysis and risk communication tool for weight of evidence evaluations and demonstration of real versus perceived risk. The results have also shown the feasibility and flexibility of this framework for use in various regulatory programs; various stages of site investigation, risk assessment and remediation; and, can be adapted for other emerging contaminants.

**3.04.P-We099 Elucidating PFAS Biomagnification: Case Study in the Finger Lakes, New York**
***N. Roxanna Razavi***[1]*, Wayne Richter*[2]*, Jesse Becker*[2]*, Yasaman Hassanzadeh*[2]*, Eric Levanduski*[1] *and Lisa B. Cleckner*[3]*, (1) SUNY College of Environmental Science and Forestry, (2) New York State Department of Environmental Conservation, (3) Hobart and William Smith Colleges*
Per- and poly-fluoroalkyl substances (PFAS), often referred to as "forever chemicals", are ubiquitous in consumer and industrial products, and now recognized to be widely present in the ambient environment. Assessing the potential PFAS contamination of important human fisheries is a global need, given the known contribution of fish as a source of PFAS to the general population and consequences for human health. Yet bioaccumulation and biomagnification of PFAS remain poorly described. Here, we use the Finger Lakes, 11 lakes within the Lake Ontario watershed, as a case study to better understand the fate of PFAS in fisheries intended for human consumption. Lake Trout (*Salvelinus namaycush*) fishing is an important economic driver in the Finger Lakes, yet Lake Trout as top predators are also susceptible to bioaccumulate specific PFAS. There remain many unknowns regarding how drivers such as food web structure and lake productivity affect PFAS bioaccumulation and biomagnification. The Finger Lakes offer a natural laboratory to investigate these questions, as they are in the same geographical region but have varying lake trophic statuses and differences in food web structure. For example, the invasive Round Goby (*Neogobius melanostomus*) is present in Cayuga Lake but not Seneca Lake. Here, we assess PFAS profiles for Lake Trout from Cayuga (n = 28), Seneca (n = 26), and Canadice (n = 22) Lakes as well as various prey species, including the Round Goby, and place these findings in context of previous results describing these lake food webs. This study will help characterize PFAS bioaccumulation and biomagnification in inland lakes of the Great Lakes watershed and inform both ecological and human health risk assessments.

**3.04.P-We100 Per- and Polyfluoroalkyl Substances (PFAS) in San Francisco Bay Waters**
***Miguel Alexander Mendez****, Martin Trinh, Ezra Miller and Rebecca Sutton, San Francisco Estuary Institute*
Per- and polyfluoroalkyl substances (PFAS) are a class of thermally and chemically stable compounds that are ubiquitous in the environment. Their widespread use in consumer, commercial, and industrial products means PFAS readily end up in waste streams that are ultimately discharged into the environment. Their highly persistent and recalcitrant nature, combined with bioaccumulation risks, raise concerns regarding potential negative impacts on human and wildlife health. Perfluorooctanoic sulfonate (PFOS) and perfluorooctanoic acid (PFOA), the best studied compounds within the class, have been identified as highly toxic with investigations linking them to a variety of adverse health effects. In this study, ambient water samples obtained from 22 sites across San Francisco Bay were assessed for the presence of 40 PFAS analytes. Across all sites, 11 unique PFAS analytes were detected with total PFAS detected ranging from ND – 30 ng/L, with a median of 6 ng/L. Perfluorohexanoate (PFHxA), PFOA, and Perfluoropentanoate (PFHpA) were the most detected analytes, though perfluorobutanoate (PFBA) and PFOS had the highest median concentrations over samples detected. Noted results will be compared to previous detections in the Bay, including wastewater and stormwater concentrations, as well as similar environments studied across the globe. Further, this study aids in advancing characterization of risks to aquatic life associated with the presence of PFAS in the Bay.

**3.04.P-We101 Fish Species Differences and Tissue Distribution of Per- and Polyfluoroalkyl Substances (PFAS) from Ashumet Pond, Cape Cod**
***Vicki Blazer***[1]*, Heather L Walsh*[1]*, Cheyenne Smith*[2]*, Steve Hurley*[3]*, Denis LeBlanc*[1] *and Andrea Tokranov*[1]*, (1)*

*U.S. Geological Survey, (2) West Virginia University, (3) Massachusetts Dept of Fish and Game*
Largemouth bass, a high trophic level fish and banded killifish, a lower trophic level species were collected from Ashumet Pond. Ashumet Pond is a natural kettle hole pond used for recreational fishing and impacted by contaminated groundwater plumes from Joint Base Cape Cod. Twenty largemouth bass were euthanized, weighed, measured and a blood sample obtained. Visible abnormalities were documented, and pieces of all tissue were preserved for histopathology. Pieces of liver and anterior kidney were preserved for molecular analyses. A piece of muscle and liver were frozen for PFAS analyses. Blood was centrifuged and plasma frozen for PFAS analyses. Twenty Killifish were euthanized, visual abnormalities documented and the whole fish preserved for histopathology. Another 20 were collected and frozen for whole body PFAS analyses. Forty PFAS were analyzed by SGS AXYS Analytical Services Ltd, British Columbia, utilizing the EPA 1633 method, an isotope LC-MS/MS method. In largemouth bass 22 compounds were detected at least once, while 18 were never detected, while in killifish there were 19 nondetects and 21 compounds detected. When comparing tissues in largemouth bass, for many compounds the highest concentrations were in the plasma, followed by liver and then muscle, however there were exceptions. These results will be compared to those of bass collected at less contaminated sites and to the biological endpoints measured.

**3.04.P-We102 Does agricultural biosolid application contaminate wetland ecosystems with per- and polyfluoroalkyl substances (PFAS)?**

***Tyler D. Hoskins**, Youn Jeong Choi, Jason T. Hoverman, Linda S. Lee and Marisol S. Sepulveda, Purdue University*

Per- and polyfluorinated alkyl substances (PFAS) are a class of >4000 chemicals that are ubiquitous environmental contaminants due to widespread use in industrial and agricultural processes. PFAS concentrate in municipal biosolids, which are often recycled as agricultural fertilizer. Numerous studies have quantified PFAS in biosolids and examined fate and transport in soils and water at application sites. Crops grown on these fields have been examined for PFAS uptake, and soil biota have been assessed for exposure and accumulation. While PFAS are known to run off from biosolid impacted fields, no studies have examined wetland ecosystems receiving this runoff. We identified a permanent, constructed wetland receiving runoff from biosolid-amended fields and a reference site with no known biosolid source. We sampled water, sediment, and biota to characterize accumulation and potential trophic transfer in our focal food webs. These included algae, aquatic invertebrates (snails, damselfly, dragonfly larvae), aquatic vertebrates including amphibian larvae, fish (bluegill, largemouth bass), and turtles (common snapping turtles, painted turtles). Fish were lethally sampled for plasma and liver, while plasma was non-lethally collected from turtles. We characterized concentrations of 50 PFAS: 20 end-products (per- and polyfluoroalkyl acids and sulfonates), 10 precursors, 12 known both as precursors and intermediate transformation products, and 8 per- and polyfluorinated alkyl ethers. Media and biota from our biosolid-impacted site had higher PFAS concentrations overall. However, PFAS were also detected in all samples from the reference site. While water and sediment at both sites were dominated by precursors and intermediates, terminal degradation products (especially perfluorosulfonic acid; PFOS) dominated in biota. Predominance of precursors in media and terminal PFAS in biota strongly suggests transformation and metabolism. Microbes and multicellular biota likely metabolize precursors and intermediates at our sites. Our data suggest that characterizing PFAS exposure at biosolid-impacted sites using water and sediment alone may be difficult. Understanding *in-vivo* PFAS metabolism and any associated toxicity is a pressing need. Given that game species (e.g., bluegill, largemouth bass) are frequently stocked and consumed by humans, biosolid-derived contamination of farm ponds represents both a human and wildlife exposure route that needs further investigation.

**3.04B PFAS and Related Compounds in Terrestrial and Aquatic Wildlife: Exposure, Uptake, Tissue Distribution, and Toxic Effects**

**3.04B.T-01 "Happy Outside, Stressed Inside" - Soybean Response to Environmentally Relevant Perfluorobutanoic acid**

***Eguono Wayne Omagamre****, Simon Zebelo and Joseph Pitula, University of Maryland Eastern Shore*

Short-chain perfluoroalkyl substances (PFAS) are generally considered to be of less environmental concern than long chain analogues due to their comparatively shorter half-lives in biological systems. Perfluorobutanoic acid (PFBA) is a short-chain PFAS with the most plant root-shoot transfer factor of all PFAS. We investigated the impact of extended exposure of soybean to irrigation water containing environmentally relevant (100 pg - 100 ng/L) to high (100 µg- 1 mg/L) concentrations of PFBA using phenotypical observation, biochemical characterization, and transcriptomic analysis. The results showed a non-monotonous developmental response from the plants, with maximum stimulation and inhibition at 100 ng/L and 1 mg/L respectively. Flavonoid levels were significantly reduced at the highest exposure level. Higher reactive oxygen species and low levels of superoxide dismutase (SOD) and catalase (CAT) were observed in all treatment groups. Differential expressions of SOD and CAT coding genes in the most stimulated and inhibited groups were not observed while non-enzymatic stress response genes and pathways were enriched in both groups with glycine betaine dehydrogenase showing the highest expression. The circadian rhythm pathway was the only differentially regulated pathway between both groups that was significantly enriched. The genes encoding for cryptochrome1 and cryptochrome2, circadian clock genes, were upregulated only in the 100 ng/L group. Several other clock genes appeared to be misregulated in the 1 mg/L group. About 18% of similarly downregulated genes in both groups are involved in the ethylene signaling pathway. Results on the potential impact of this observation in terms of soybean response to PFBA co-exposure with other abiotic stressors i.e., heat, salinity and drought will be presented. We conclude that PFBA induced stress on soybean plants similar to long chain PFAS, and the observed hormetic stimulation may be an overcompensation response, via the circadian rhythm pathway, to the induced stress.

**3.04B.T-02 Comparison of the Sub-lethal Metabolic Response of Daphnia magna to Per- and Polyfluoroalkyl Substances (PFAS) of Varying Chain Length**

***Lisa Labine***[1]*, Erico Oliveira Pereira*[1]*, Sonya Kleywegt*[2]*, Karl Jobst*[3]*, Andre Simpson*[1] *and Myrna Simpson*[1]*, (1) University of Toronto, Canada, (2) Ministry of the Environment, Conservation and Parks, Canada, (3) Memorial University of Newfoundland, Canada*

Per- and polyfluoroalkyl substances (PFAS) are a well-known class of persistent organic pollutants that are ubiquitously found in aquatic environments. While legacy 8-carbon chain PFAS have been well-studied, alternative PFAS concentrations are exceeding that of legacy pollutants. Despite their widespread detection, there is inadequate information surrounding their sub-lethal toxicity and mode of action at the molecular level to aquatic model organisms, such as *Daphnia magna*. Perfluorobutanoic acid (PFBA), perfluorohexanoic acid (PFHxA), perfluorohexanesulfonic acid (PFHxS), and perfluorononanoic acid (PFNA), are four widely detected PFAS alternatives of varying physicochemical properties. This study examines the sub-lethal metabolic response of PFAS with varying chain length and functionality to *D. magna* using mass spectrometry (MS)-based metabolomics. Following acute 48 h exposure, the polar metabolite profile was extracted from a single daphnid was analyzed using a targeted experimental design that quantifies up to 52 endogenous metabolites. Multivariate analyses demonstrated significant ($p < 0.05$) separation between sub-lethal concentrations of PFHxA, PFHxS, and PFNA relative to the unexposed controls. However, no significant ($p < 0.05$) separation was observed with PFBA exposure. Univariate analyses revealed that several amino acids (arginine, cysteine, histidine, glycine, phenylalanine, proline, and threonine), nucleot(s)ides (GMP, guanosine, AMP, IMP, and uridine), and neurotransmitters (acetylcholine and choline) concentrations were significantly ($p < 0.05$) perturbed with individual PFAS exposure. Pathway analysis uncovered significant disruptions in biochemical processes associated with amino acid metabolism, energy generation, and protein synthesis. Differences in the metabolic responses of different PFAS at the selected concentrations demonstrate that the inherent chemistry of each PFAS (chain length and polar functional group) plays a unique role in the observed metabolic perturbations. These results provide evidence that while PFAS chemistry invokes unique responses in *D.*

*magna*, there is also an underlying toxic mode of action that is common with PFAS exposure. This work highlights the importance of observing the molecular-level response of different pollutants within the same chemical class, in addition to conventional toxicity endpoints.

**3.04B.T-03 Sublethal Toxicity of Diverse PFASs to Three Freshwater Invertebrates: Comparisons Among Species, Structures, and Mixtures**

***Sarah Kadlec***[1]*, Will Backe*[1]*, Russell Erickson*[1]*, Leah Glimsdal*[1]*, Russ Hockett*[1]*, Sarah Howe*[2]*, David Mount*[1]*, Ian Mundy*[1]*, Edward Piasecki*[2]*, Henry Sluka*[1] *and Lauren Votava*[1]*, (1) U.S. Environmental Protection Agency, (2) Oak Ridge Institute for Science and Education (ORISE) participant at U.S. Environmental Protection Agency*

The majority of experimental data on the ecological effects of per- or poly-fluorinated alkyl substances (PFASs) are for a small number of compounds within the structurally diverse set of PFASs whose potential ecological risks are of interest. There is a clear need for a systematic understanding of how PFAS toxicity varies across chemical structures and species. To address this need, we have generated aquatic toxicity data for 16 PFASs among the perfluorinated sulfonate, perfluorinated carboxylate, perfluorinated sulfonamide, and fluorotelomer classes, with chain lengths of 3 to 10 fluorinated carbons. Toxicity tests were conducted with three freshwater invertebrate species (*Ceriodaphnia dubia, Chironomus dilutus,* and *Hyalella azteca*) using 7-d exposures that allowed measurements of sublethal endpoints (growth or reproduction). Sublethal effect concentrations varied widely: some chemicals caused no effects up to 200 mg/L, whereas others reduced growth by 50% in the 0.01 mg/L range. Among all PFAS classes, toxicity generally increased with increasing chain length. A notable exception was toxicity of perfluorinated sulfonates to *C. dilutus*, which exhibited the greatest sensitivity to PFOS and PFNS (8 and 9 carbons, respectively), and were less sensitive to PFASs having shorter (<8 carbons) or longer (>9 carbons) chain lengths. Perfluorinated sulfonates were dramatically more toxic to *C. dilutus* than were perfluorinated carboxylates, by as much as three orders of magnitude. In strong contrast, those two classes had similar effect concentrations and relatively low toxicity to *C. dubia* and *H. azteca* ($EC_{50}$ values in the 1 to 200 mg/L range). Comparing across PFASs with similar chain lengths, perfluorinated sulfonamides were the most toxic class to all three species. This presentation discusses how structure-toxicity patterns observed among and between species facilitate hypothetical groupings of compounds with common mechanism of action and prioritization of compounds/species for further studies involving body burden and mixture toxicity. We also present results of binary mixture studies to characterize toxic interactions and infer similar/dissimilar mechanisms. *The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the United States Environmental Protection Agency.*

**3.04B.T-04 Feeding Ration Impacts on Larval Pimephales promelas Growth Endpoint After a 7-Day Subchronic Perfluorooctane Sulfonic Acid (PFOS) Exposure**

***Jonna Boyda***[1]*, Alan Kennedy*[1]*, David Mount*[2]*, Paige Krupa*[3] *and Tom Biber*[3]*, (1) U.S. Army Corps of Engineers, (2) U.S. Environmental Protection Agency, (3) U.S. Army Engineer Research and Development Center*

The larval (<48 hours old) fathead minnow, *Pimephales promelas*, 7-day subchronic survival and growth (e.g. mass) standard toxicity test method is commonly used for research and regulatory testing. Fish growth is affected by factors such as density, feeding ration, and feeding frequency, and existing guidelines regarding feeding methodology during this test are general and open to interpretation, specifically regarding the latter two factors. This introduces inter-test and inter-laboratory variability; such data variability can substantially impact downstream regulatory decisions and clean up thresholds. The current study was conducted in two parts: 1) a control portion to determine the most significant feeding factors to maximize *P. promelas* larval growth with laboratory logistics considered; and 2) a case study on how the most significant feeding factors impact *P. promelas* survival and growth during a 7-day subchronic Perfluorooctane Sulfonic Acid (PFOS) exposure. The control optimization study supported that feeding frequency ($p = 0.0006$), but more importantly feeding ration ($p < 0.0001$), were significant factors in fish growth (mass). In the subsequent PFOS study, fish were fed either

a high or low ration of *Artemia* (e.g., 1,000 or 500 nauplii) twice daily, in the morning and late afternoon, while nominally exposed to 0.1 to 5mg/L of PFOS. Statistics determined the fish that were fed the lower ration of *Artemia* had significantly ($p < 0.0001$) lower mass in each exposure concentration (and control), apart from 5 ppm, where survival was the lowest, compared to the fish fed the high ration of *Artemia*. Interestingly, the IC50 value for the low ration group was 1.7 mg/L, while the high ration group IC50 was 0.7 mg/L. Survival was not significantly different between the low ration and high ration groups, but the nominal LC50 for the low ration fish group was 13% lower than the high ration fish group. The outcome of this study allows for scientists to apply a more prescriptive feeding methodology for the 7-day *P. promelas* subchronic test, where larval growth will not be limited by feeding ration and will result in more consistent and repeatable growth during chemical exposures.

**3.04B.T-05 PFAS and other contaminants of emerging concern in a Great Lakes urban-dominant watershed: implications for human and ecosystem health**

***Tracie R Baker***[1]*, Bridget B. Baker*[1]*, Alex Haimbaugh*[2]*, Supriya Gupta*[3] *and Serena George*[4]*, (1) University of Florida, (2) Wayne State University, (3) University of Michigan, (4) University of Wisconsin, Madison*

Within the Great Lakes, contaminants of emerging concern (CECs) have been detected in urban surface waters. Understanding the human and ecological health consequences of environmentally-relevant exposures to persistent CECs, particularly as complex mixtures, is essential. The Lake Huron to Erie corridor (HEC) is an Area of Concern, the drinking water source for ~4 million people, hub of >30% of Michigan's fishing effort, and home to vital habitats. Surface water and sediment samples were collected over three years at multiple locations within HEC to investigate more than 150 CECs, including pharmaceutical and personal care products (PPCPs), pesticides, and per- and polyfluoroalkyl substances (PFAS). Sediment was only analyzed for PFAS. More than fifty compounds were detected at ng/L or ng/kg levels., We then analyzed the concentration of 40 PFAS from muscle and serum samples of sport fish representing different trophic levels along HEC to assess PFAS bioaccumulation and potential human-health implications. We have used this data to inform follow-up studies in the laboratory zebrafish model and are evaluating changes in embryonic development, behavior, reproductive capacity, sex ratio, offspring survival, transcriptome, and transgenerational health. This multi-pronged approach is aimed at informing human and ecosystem health, water treatment/sewage infrastructure, remediation/restoration efforts, natural resource management, public education, and community revitalization.

**3.04B.T-06 Integration of Multi-omics Reveals the Commonality of Neurotoxicity Caused by PFOS and PFBS in Zebrafish Larvae**

***Eun Ki Min****, HoHyun Jin, Ji Yoon Kang and Kitae Kim, Seoul National University of Science and Technology, Korea, Republic of (South)*

The differential biomolecular mechanisms between legacy per- and polyfluoroalkyl substances (PFAS) and their short-chained alternatives were studied at the same concentrations, which hampers our understanding of the potency of alternatives. We therefore compared the commonalities of PFOS and PFBS with profiled biomolecules in zebrafish larvae such as transcripts, proteins, and metabolites. PFBS showed approximately 2 orders of magnitude lower developmental toxicity than PFOS based on the benchmark dose (BMD) of embryonic mortality. Although the toxicity potency was different, larval neurobehavior patterns were similar; hyperactivity and constant larval movement was observed at the same ratio of concentrations on a $BMD_5$. Individual omics analysis was conducted to obtain biomolecular profiles of transcripts, proteins, and metabolites at concentrations determined by the proportion of BMD between PFOS and PFBS, which well explains comparable neurobehavior alterations involved in oxidative stress, immune response, lipid metabolism, and energy metabolism. Knowledge-based integration of multi-omics was further applied to better explore the underlying neurotoxicity mechanism of PFOS and PFBS. The integrated multi-omics analysis revealed the commonality in enriched pathways affected by both PFOS and PFBS such as dysregulation of various signaling pathways, lipids metabolism, amino acid metabolism, and oxidative response. In particular, the biomolecular mechanism of PFOS and PFBS was closely interlinked, including calcium signaling pathway, primary bile acid biosynthesis, and peroxisome. These findings implied that PFBS could be an alternative of PFOS because of a low toxicity in developing zebrafish embryos. However, we suggested that the potential risk of PFBS should also be carefully managed based on the similarly dysregulated biomolecular mechanisms between PFOS and PFBS as elucidated by individual and integrated multi-omics analysis.

### 3.04B.T-07 In Vitro Characterization of the Emerging Perfluoroalkyl Substance Replacement, Perfluoroethylcyclohexane Sulphonate (PFECHS)

***Hannah Mahoney****, Yuwei Xie, Jenna Cantin, Markus Brinkmann and John Giesy, University of Saskatchewan, Canada*

The widespread application of poly- and per-fluoroalkyl substances (PFAS) has resulted in some substances being ubiquitous in environmental matrices, and their resistance to degradation have allowed them to accumulate in wildlife and humans with associated toxic effects. While certain substances of concern have been phased-out or banned, new substances continue to be produced. One such substance is perfluoroethylcyclohexane sulphonate (PFECHS), an analogue of perfluoroctanesulphonic acid (PFOS) which has recently been detected in multiple environmental media around the globe. However, there is little information on the toxic potency of PFECHS and other cyclic-PFAS in general. Therefore, this research aimed to characterize PFECHS and elucidate its effects in the aquatic environment using *in vitro* techniques. Liver cell lines RTL-W1 and ZFL-4 was used to predict the exposure response of PFECHS in rainbow trout, and further analyses focused on membrane effects were completed with rainbow trout leukocytes. PFECHS does not result in apical adverse effects at environmentally relevant concentrations apart from decreasing plasma membrane polarity. While molecular alterations were also observed at exposure concentrations lower than those that induce apical effects, chemical analyses of the exposure media have further supported PFECHS is not as acutely potent, nor as potentially bioaccumulative as legacy congeners. Determining the toxic potency of PFECHS is an important step in determining the safety of potential PFAS replacements, and such research will help better inform the viability of replacements as a strategy for PFAS management in the future.

### 3.04B.T-08 Using Fatty Acids and Stable Isotopes to Assess the Flow of Perfluoroalkyl Substances in a linked Aquatic-Terrestrial Avian Food Web

***Kailee Hopkins***[1]*, Melissa McKinney*[1]*, Robert J. Letcher*[2]*, Amandeep Saini*[2]*, Natalie K. Karouna-Renier*[3] *and Kim J. Fernie*[2]*, (1) McGill University, Canada, (2) Environment and Climate Change Canada, (3) U.S. Geological Survey*

Limited research has been conducted on the flow of per- and polyfluoroalkyl substances (PFAS), including perfluoroalkyl sulfonates (PFSAs), perfluoroalkyl carboxylates (PFCAs), their precursor compounds, and newer alternative PFAS, through aquatic and terrestrial food webs. As a species that forages on terrestrial and aquatic invertebrates, tree swallows (*Tachycineta bicolor*) potentially transfer and accumulate PFAS from aquatic and terrestrial ecosystems. Our objectives were to assess PFAS flow in a linked aquatic-terrestrial tree swallow food web, by 1) determining food web structure using fatty acid (FA) signatures and stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values, 2) investigating bioaccumulative PFAS levels and patterns across abiotic and biotic food web compartments, 3) modelling the influence of diet on PFAS concentrations in tree swallows, and 4) estimating the bioaccumulation and biomagnification of target PFAS in this food web. We characterized 8 PFSAs, 13 PFCAs, and 25 precursor compounds in air, water, sediment, aquatic invertebrates, terrestrial invertebrates, and tree swallow gastrointestinal tracts and livers. Aquatic and terrestrial invertebrates showed distinct FA signatures, with higher proportions of long-chain polyunsaturated fatty acids in aquatic invertebrates, typical of aquatic biota (e.g., 22:5n3 and 22:6n3). Tree swallow FA signatures were intermediate between aquatic and terrestrial invertebrates, consistent with reliance on both food sources. Tree swallow dietary patterns inferred by $\delta^{13}C$ and $\delta^{15}N$ also confirmed utilization of both terrestrial and aquatic food sources. There were clear differences and similarities in PFAS profiles among ecosystem compartments. FTOHs dominated air profiles, while short-chain PFSAs and PFCAs were prominent in water. Sediment and aquatic invertebrates showed more of the long-chain PFSAs and PFCAs and the precursor 5:3 FTCA. Only PFOS, and much lower concentrations of 6:2 FTS, were detected in terrestrial invertebrates. PFOS and 5:3 FTCA were also major PFAS in tree swallow GI tracts and livers, but like the water and aquatic invertebrates, these tissues contained numerous other PFASs. These patterns are consistent with dietary uptake of PFAS by tree swallows via aquatic and terrestrial pathways. Modelling of diet influences, as well as bioaccumulation and biomagnification of PFAS in this food web, are also being examined.

## 3.04B.P PFAS and Related Compounds in Terrestrial and Aquatic Wildlife: Exposure, Uptake, Tissue Distribution, and Toxic Effects

### 3.04.P-Th051 Active Biomonitoring for PFAS using the invasive Asian clam in the Occoquan River Watershed

***Lauren Koban***, *Thomas Huff and Amy Fowler, George Mason University*

Recent monitoring efforts found high concentrations of Per- and Polyfluoroalkyl Substances (PFAS) in Northern Virginia drinking water sourced from the Occoquan River. The Occoquan watershed includes potential PFAS sources from industrial facilities, airports, military bases, municipal wastewater treatment plants, and non-point sources such as stormwater.

This study tests the reliability of using the invasive Asian clam *Corbicula fluminea* as a bioindicator for PFAS in surface water and sediment within the Occoquan River watershed. *Corbicula fluminea* are invasive, abundant bivalve mollusks and sedentary filter feeders that live at the water-sediment interface in aquatic environments. They are known for high filtration rates and tolerance of toxicants. We hypothesize PFAS are primarily conserved in *Corbicula fluminea* tissue, which, combined with sediment and water measurements, will aid in identifying recent and ongoing sources of PFAS in the Occoquan watershed.

In situ sampling locations were grouped into three areas: (1) upstream from a regional water reclamation plant, (2) between a reclamation plant and a drinking water treatment plant, and (3) downstream from the drinking water treatment plant.

Initial sampling occurred in September 2021 within the same area, with results informing in situ study sites. Surface water samples contained high concentrations of 6:2 fluorotelomer sulfonate (6:2 FTS) in the most upstream sampling location, indicating a potential contamination gradient. Sediment and clam analysis is still ongoing. Concentrations of the chemicals across matrix levels (i.e., water, sediment, clams) will be compared to confirm if a gradient exists and if data is correlated between mediums. This study also aims to determine if using *Corbicula fluminea* is more cost and resource-efficient than other PFAS sampling methods.

### 3.04.P-Th053 Examining Effects of an Estrogenic PFAS, 1H,1H,8H,8H-Perfluorooctane-1,8-diol (FC8-diol), Using the Fathead Minnow Ecotoxchip

***Kelvin Santana Rodriguez***, *Daniel L. Villeneuve, Jenna E. Cavallin, Brett R. Blackwell, Rachel Hofer, Michael Kahl, Emma Stacy, Gerald Ankley, Robin Kutsi, Kathleen Jensen, John Hoang and Mackenzie Morshead, U.S. Environmental Protection Agency*

Per- and polyfluoroalkyl substances (PFAS) are of concern due to their persistence in the environment and potential health effects to both human and wildlife. However, for many PFAS there is very limited or no toxicity information related to the modes of action in aquatic species. In a previous in vivo study, adult male fathead minnows (*Pimephales promelas*) were exposed via the water for four days to an ER-active PFAS; 1H,1H,8H,8H-Perfluorooctane-1,8-diol (FC8-diol). Expression of four genes known to be affected by estrogen exposure were evaluated in liver tissue using quantitative real time polymerase chain reaction (QPCR). Results showed that 0.15-1.5 mg FC8-diol/L caused an upregulation of *vtg* and *esr1* expression and a downregulation of *igf1* and *apoeb* similar to that caused by 1.0 µg 17β-estradiol/L (positive control). The purpose of this follow-up study was to examine whether a broader evaluation of gene expression might reveal additional mode(s) of action for this compound. To do this, samples were further evaluated using EcoToxChips, a PCR array-based approach that allows for simultaneous measurement of over 375 species-specific genes of toxicological interest. A total of 25 fathead minnow liver RNA samples (5 samples from each test concentration; 0, 0.015, 0.047, 0.15 and 0.47 mg FC8-diol/L) from the original in vivo study were evaluated using the fathead minnow EcoToxChip v. 1.0. Resulting data were analyzed with the online EcoToxXplorer tool (EcoToxXplorer.ca). Among the genes analyzed, 58 and 60 were significantly up- and down-regulated respectively by one or more FC8-diol treatments. Previous QPCR results were further confirmed, with the EcoToxChips showing an upregulation of *vtg* and *esr1* and downregulation of *igf1* (*apoeb* is not present on the chip). Additional genes related to estrogen receptor activation like *esr2a* and *esrrb* were also affected providing further confirmation of the estrogenic nature of FC8-diol. Furthermore, genes such *as lpl, hmgcra, acsl5, acss2, acadvl, dgat2* and *scd* that are involved in additional biological pathways related to fatty acid degradation and lipid metabolism were significantly affected. These results both add confidence in the use of EcoToxChips as a general tool for inferring chemical mode(s) of action and provide some further insights as to possible biological effects of FC8-diol. *The contents of this presentation neither constitute, nor necessarily reflect, US EPA policy.*

**3.04.P-Th055 Determination of Bioconcentration Factors for Per- and Polyfluorinated Alkyl Substances in Aquatic Invertebrates.**

***Ian Mundy***[1]*, Will Backe*[1]*, Leah Glimsdal*[1]*, Sarah Howe*[2]*, Sarah Kadlec*[1]*, David Mount*[1]*, Edward Piasecki*[2]*, Henry Sluka*[1] *and Lauren Votava*[1]*, (1) U.S. Environmental Protection Agency, (2) Oak Ridge Institute for Science and Education (ORISE) participant at U.S. Environmental Protection Agency*

Per- and polyfluorinated alkyl substances (PFAS) have elicited research interest in recent years, owing to their near ubiquitous environmental distribution, recalcitrance to degradation, and demonstrated ability to bioconcentrate in plants and wildlife. A subset of this research has focused on PFAS distribution and bioconcentration in high trophic level organisms, often those consumed by humans (e.g. fish). Yet, commensurate effort has not been devoted to bioaccumulation assessments in lower trophic level organisms. In addition to providing input data to food web modeling, uptake by low trophic level organisms is also of interest as a component in our group's research into water column toxicity of PFAS with small aquatic invertebrates. Within this work, measured bioconcentration factors (BCFs) can be used to relate water column effect concentrations to expected internal dose. However, the lack of relevant measured BCFs leaves this line of analysis unavailable. This presentation focuses on experiments to measure PFAS BCFs in *Hyalella azteca*, a small aquatic invertebrate used by our group for sublethal toxicity testing of PFAS. The initial focus is primarily on legacy PFAS within the homologue series of sulfonic and carboxylic acids. Analyses of tissue residues and water concentrations are made primarily by HPLC-MS, with HPLC-MS/MS used as needed. Method development efforts have yielded a stream-lined procedure for tissue extraction and clean-up, consisting of homogenization and extraction with acetonitrile followed by centrifugation and dilution prior to analysis. Preliminary measurements in single adult *H. azteca* have found BCFs (L/kg-wwt) of $7.0 \pm 1.5$, $62 \pm 12$, and $26 \pm 6$ for perfluorohexane sulfonate, perfluorooctane sulfonate, and perfluorononanoic acid, respectively. Future work will expand this scope to other, precursor PFAS and additional organisms. This will also include investigation of other factors that may influence BCF, such as testing with juvenile versus adult organisms and determination of BCF in mixtures versus single-chemical tests.

**3.04.P-Th056 Investigating the Role of Coastal Wetland Filtration on Per- And Polyfluoroalkyl Substances (PFAS) Contamination**

***Ashley Pavia****, Shannon Jones and Miling Li, University of Delaware*

Per- and polyfluorinated alkyl substances (PFAS) are a group of anthropogenic chemicals that have been linked to adverse toxicological and ecological effects in both wildlife and humans. Few studies have assessed the fate and transport of PFAS in coastal environments and the effectiveness of coastal wetland filtration for sequestering PFAS. Due to the water-solubility of PFAS, groundwater may be a potential source of PFAS contamination to the coastal oceans. Saltmarshes act as a filter for many contaminants but their role in mediating PFAS transport is unknown. The goal of this study is to investigate how geochemical and hydrological conditions affect PFAS fate and transport and to assess the effectiveness of coastal wetland filtration of PFAS. We use St. Jones Reserve, a tidal marsh positioned between a military base and Delaware Bay as the study site. We will compare the levels and composition of PFAS in this wetland to its upstream tributaries and downstream Delaware Bay to evaluate the ability of coastal wetlands for retaining PFAS. We collect groundwater, porewater, surface water, and sediment from various sites with contrasting geochemical and hydrological conditions in the marsh. The results will help determine the factors affecting PFAS distribution in saltmarshes. This study will shed light on the role of coastal wetlands in PFAS sequestration and supply to the ocean.

**3.04.P-Th057 The Impact of Perfluorooctane Sulfonate on Photosynthesis and Cellular Processes: Insight from the Diatom, Thalassiosira pseudonana.**

***Sarah Davis**, Manoj Kamalanathan, Shaley Klumker, Christian Pryor, Alexis Mitchell and Antonietta Quigg, Texas A&M University at Galveston*

Perfluorinated alkylated substances (PFASs) are a group of synthetic chemicals that have experienced widespread use in industrial and commercial products in the last half century. In recent decades the PFAS, perfluorooctane sulfonate (PFOS), has been identified as toxic, bioaccumulative, and persistent, and is now found in terrestrial and aquatic ecosystems across the globe. Though phytoplankton photosynthesis plays an integral role in fueling aquatic systems, a limited number of studies have investigated the toxicity of PFOS on microalgal photosynthesis, resulting in an incomplete understanding of its ecotoxicity on aquatic primary producers. This research evaluated the impact of PFOS [0, 2, 4, 8, 16, 24 mg/L (ppm)] on the growth and photosynthetic efficiency of the marine diatom, *Thalassiosira pseudonana*, over a 6-day period. Monitoring of each parameter was conducted using classical cell counting procedures (growth) and fluorometry (photosynthetic efficiency). During the exposure period, *T. pseudonana* experienced significant decline ($p < 0.05$) in the health of photosystem II even at low exposure concentrations, resulting in a correlation between increasing PFOS concentrations and lowered photosynthetic efficiency. This study will be replicated to include examination of lipid peroxidation using lipid content and malondialdehyde (MDA) to further assess the disruption of cellular processes due to PFOS. We hypothesize that the decline in photosynthetic efficiency will increase cellular reactive oxygen species, resulting in higher concentrations of the lipid peroxidation byproduct, MDA, and lowered lipid content per cell. This is the first study assessing *T. pseudonana* response to PFOS and reveals *T. pseudonana* is more vulnerable to PFOS exposure compared to other evaluations of phytoplankton species. This may indicate a species-specific vulnerability to PFOS exposure.

**3.04.P-Th058 Considerations for the Use of Zebrafish Toxicity Data for PFOS Aquatic Life Criteria Derivation**

***Jason M. Conder**, Geosyntec Consultants, Inc.*

Zebrafish (*Danio rerio*) is among the most sensitive aquatic species to perfluorooctane sulfonate (PFOS). Effect benchmarks from laboratory zebrafish PFOS toxicity studies in PFOS-spiked water strongly influence PFOS aquatic life criteria calculated by environmental regulatory agencies and other researchers. This presentation will present a review of PFOS effects benchmarks for lethality, growth, and reproduction endpoints from more than 20 zebrafish toxicity studies, including a recent multi-generational study (currently in peer review) conducted by the US Army Corps of Engineers Engineer Research & Development Center (ERDC) and the Sediment Management Work Group, in collaboration with the US Environmental Protection Agency (USEPA). Review results from most acute studies (approximately 7 days or less) indicate LOEC (lethality, malformations) and EC50 values for PFOS are generally greater than 1,000 µg/L. Twelve key studies examining longer exposures (including multigenerational exposures of 300 days or more) indicate chronic thresholds that are one order of magnitude lower. For example, the multi-generational ERDC study found that 100 µg/L resulted in highly variable impacts on mortality, ranging from 0% to 40% among the three generations evaluated (no significant effects on growth or reproduction were observed). This result was consistent with 10 of the other 11 chronic zebrafish studies, which indicated LOEC values (various endpoints) in the 40 to 400 µg/L range. The study that noted an exception to this general observation (Keiter et al., 2012) found a 5-10% reduction in growth at exposures as low as 0.6 µg/L. This study was excluded from calculations conducted for USEPA's April 2022 draft ambient water quality chronic criterion for PFOS in freshwater, primarily because it was limited in scope (only 3 PFOS dose levels and only 2 replicates per dose) and limited in measurement of PFOS in exposure water. Given the limitations of the Keiter et al. (2012) study and the 2- to 3-order of magnitude difference between its 0.6-µg/L threshold value and values from the other chronic studies, the Keiter et al. (2012) study should not be quantitatively included in criteria calculation or site-specific decisions to protect fish. Overall, the weight of evidence indicates that that the thresholds for ecologically relevant chronic effects of PFOS in fish are in the 40 to 400 µg/L range.

**3.04.P-Th059 Spatial and temporal trends of perfluoroalkyl substances (PFAS) in seabird eggs from the Pacific coast of Canada**

***Robert Kesic***[1]*, John E. Elliott*[2]*, Kyle Elliott*[3] *and Sandi Lee*[2]*, (1) Simon Fraser University, Canada, (2) Environment and Climate Change Canada, (3) McGill University, Canada*

Seabirds are useful indicators for monitoring levels of legacy and emerging contaminants in marine ecosystems because they feed at relatively high trophic levels, suitably integrating contaminant exposure across space, time, and communities. For decades, seabird eggs have been used as a non-invasive sampling matrix to monitor contaminant exposure and biomagnification processes in marine food webs because they are: 1) easy to identify and collect, 2) easy to homogenize and analyze, and 3) representative of the contaminant burden in the female adult at the time of laying. In light of regulations and restrictions on the production and use of PFAS in North America, we set out to investigate spatial and temporal trends (1990/1991 – 2018/2019) of 13 PFAS in the eggs of five seabird species (double-crested cormorants, pelagic cormorants, rhinoceros auklets, ancient murrelets, and Leach's storm-petrels) breeding off the Pacific coast of British Columbia, Canada. PFOS (perfluorooctane sulfonate) dominated the PFAS profile in the eggs of all species, comprising an average of more than 60% of ΣPFAS in each sampling year. In some species (i.e. auklets and storm-petrels), PFOS concentrations peaked in the late 1990s and early 2000s before levelling off. By contrast, compounds such as PFCA (perfluoroalkyl carboxylates) and PFUdA (perfluoroundecanoate) showed increasing temporal trends in some species. The use of stable isotope analyses of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ for explaining the observed patterns will be discussed further.

**3.04.P-Th060 Ecotoxicity and Accumulation of Perfluorononanoic Acid (PFNA) in Fathead Minnows and an Approach to Developing Protective Thresholds in the Aquatic Environment Through Species Sensitivity Distribution**

***Jamie Suski***[1]*, Christopher J. Salice*[2]*, Michael Chanov*[1]*, Chris Heron*[3] *and Jennifer Field*[3]*, (1) EA Engineering, Science, and Technology, Inc., (2) Towson University, (3) Oregon State University*

Perfluorononanoic acid (PFNA) is a perfluoroalkyl carboxylic acid (PFCA) with a nine-carbon chain structure. Like the more widely studied per- and polyfluoroalkyl substances (PFAS), perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), PFNA shares characteristics of hydro- and oleophobicity and is a terminal PFAS. Historically, PFNA was commonly used in many industrial processes and is regularly found in environmental media at PFAS impacted sites. The aim of this research is to explore the impacts and accumulation of PFNA in the fathead minnow over critical life-stages. Measured exposure concentrations were 0, 125, 250, 500 and 1000 µg/L. There were no toxicological impacts of PFNA quantified in adult fish at any exposure concentration. However, growth (as measured via biomass) was reduced in larval fish (F1) at exposures ≥ 250 µg/L. Furthermore, we have measured PFNA in gonads, brain and liver that demonstrates accumulation in a dose-dependent fashion. In comparison with other aquatic receptors, the fathead minnow is fairly sensitive. A species sensitivity distribution including these data and those collected from the literature results in a PFNA HC5 of 0.046 mg PFNA/L. To our knowledge this is the first study aimed at identifying PFNA thresholds for ecological screening-level criteria.

**3.04.P-Th061 Uptake and elimination of per- and polyfluoroalkyl substances in earthworms exposed to amended artificial soil**

***Guilherme R. Lotufo***[1], *Robert Boyd*[2], *Michael Simini*[3], *Roman Kuperman*[3], *Jennifer Guelfo*[4] *and Zhao Yang*[4], *(1) U. S. Army Corps of Engineers, (2) U.S. Army Engineer Research and Development Center, (3) U.S. Army DEVCOM Chemical Biological Center, (4) Texas Tech University*

We investigated the uptake and elimination kinetics of per- and polyfluoroalkyl substances in earthworms exposed to amended artificial soil to derive bioaccumulation factors (BAF). Uptake kinetics was determined at two concentrations, 0.01 and 1 mg/kg, of each of 18 PFAS relevant to the DoD (8:2 FTS, PFBA, PFBS, PFDA, PFHpA, PFHpS, PFHxA, PFHxS, PFNA, PFOA, PFOS, PFOSA, PFPeA, PFTeDA, PFTrDA and PFUdA). Exposed earthworms were sampled in triplicate after 1, 3, 7, 14, 21 and 28 d. The concentration of PFAS remained relatively unchanged during the exposure and rates of uptake were estimated for most individual PFAS. For the 0.01 mg/kg treatment, steady state was reached (based no significant difference between the last three endpoints) for all PFAS except PFHpS, PFHxS, PFOS, PFTeDA (most tissue samples below detection), and PFTrDA. For the 1 mg/kg treatment, steady state was reached for all PFAS except 8:2 FTS, PFDA, PFTeDA, and PFUdA. Reliable rates of uptake were obtained from all PFAS except for PFHxA and PFPeA. Uptake rates were lower for the 1 mg/kg (typically by a factor of 3 to 4) for all individual PFAS except 8:2 FTS, likely because of sublethal physiological impairment caused by the exposure to a high concentrations of sum PFAS (i.e., approximately 10 mg/kg) in the exposure soil. For the 0.01 mg/kg treatment, BAFs ranged from 4 to 49 for most PFAS, but were higher for PFHxS (72), PFTeDA (271), and PFUdA (299). BAFs were lower for the 1 mg/kg treatment for all individual PFAS, typically by a factor ranging from 3 to 16. We are determining the elimination kinetics in the ongoing studies and will report the results at the meeting. Elimination rates will be used to calculate kinetically derived BAF values as the ratio of uptake and elimination rate constants.

**3.04.P-Th062 Role of salinity in the differential regulation of transporters by PFOS in Fundulus heteroclitus**

***Tyler T. Davis*** *and William S. Baldwin, Clemson University*

Perfluorooctane sulfonate (PFOS) is a persistent organic pollutant used in the making of water repellent textiles, wax, pesticides, and flame retardants. Because of its persistence, it is a common pollutant that bioaccumulates in both freshwater and saltwater ecosystems. The purpose of our current study was to investigate the potential role of the gills in adapting to PFOS exposure and aiding in its elimination. The gills are a key area of uptake and elimination of many chemicals from water but work differently in freshwater fish than in saltwater fish, especially the uptake of key ions. *Fundulus heteroclitus* (sometimes called mummichog or killifish) are estuarine fish with high resiliency to changes in salinity. Therefore, we adapted this brackish species to freshwater and investigated how different salinities alter transporter expression following PFOS exposure. Saltwater and freshwater adapted mummichog were exposed for 0, 2, 10, or 20 days to PFOS at 0, 0.01, 1, or 100 ppb. RNA was extracted and gene expression was determined by qPCR. Freshwater-adapted mummichogs show greater expression of the organic anion transporter 1 (OAT1) and Na/K-ATPase1a1 with similar expression in the glucocorticoid receptor (GR) and P-glycoprotein (P-gp). GR and CFTR were altered by PFOS. GR expression is repressed by PFOS in saltwater at all concentrations tested but induced by only 100 ppb PFOS in freshwater adapted mummichog. Cystic fibrosis transmembrane conductance regulator (CFTR) was induced by 100 ppb PFOS only in freshwater adapted mummichog. CFTR shows differential expression between the different fresh/saltwater adapted PFOS-exposed groups because its expression only changed in freshwater adapted fish in a concentration-dependent manner. In summary, PFOS alters the expression of several distinct transporters in the gills of mummichog with salinity differences in response. With the exception of GR which was highly sensitive in the saltwater adapted mummichog, more genes were responsive in freshwater adapted fish. Currently, we are assessing bioconcentration of PFOS in the multiple tissues such as the liver, gills, and skeletal muscle by liquid chromatography/mass spectrometry (LC/MS) to associate transporter expression with PFOS concentrations, determine bioconcentration factor (BCF) and whether BCF differs between freshwater and saltwater adapted fish.

**3.04.P-Th063 Hexafluoropropylene Oxide Dimer Acid (GenX) Reproductive Toxicity and Neurotoxicity in Caenorhabditis elegans**

***Storm Cash*** *and Xiaoping Pan, East Carolina University*

Per- and polyfluoroalkyl substances (PFAS) are commercially manufactured chemicals that are used in a wide range of industries. Some PFAS have been banned due to past research indicating negative health impacts and bioaccumulation in humans and the environment. GenX (hexafluoropropylene oxide dimer acid (HFPO-DA)) is a short-chained replacement for perfluorooctanoic acid (PFOA), but little research has been conducted to evaluate the potential health impacts of GenX. *Caenorhabditis elegans* (*C. elegans*) were used to assess the impact of GenX exposure on neurological behaviors, reproduction, and their mechanisms. Age-synchronized *C. elegans* were dosed in a range from 14 to 71,000 ng/L, which represent environmentally relevant concentrations of GenX. Results indicate that GenX exposure increases pharyngeal pumping rate and head thrashing rates of *C. elegans*, notably at low dosage levels. GenX exposure also caused the increased production of offspring in a dose-specific manner with germ cell counts in various gonadal regions increasing. Gene expression analysis indicated significant gene expression suppression following GenX exposure in certain genes related to oxidative stress, oogenesis, and spermatogenesis. Testing into potential mitochondrial toxicity is currently ongoing. These results align with past research concerning excitatory responses in *C. elegans* and certain vertebrate species caused by PFOA. Additionally, the results provide new insight into GenX toxicity.

**3.04.P-Th065 Developmental Impacts of Hypersalinity and Per- and Polyfluoroalkyl Substances (PFAS) on Early Life Stage Red Drum**

***Kathleen Roark****, Kerri Lynn Ackerly and Kristin Nielsen, The University of Texas at Austin*

Estuaries are important sites for many biological and ecological processes, including acting as a nursery habitat for fishes. Freshwater inflow from terrestrial sources and tidal inflow from the ocean cause salinity fluctuations and gradients that estuarine biota must tolerate. Changes in flow regimes can result from anthropogenic activity in coastal watersheds and via rising temperatures related to climate change. Salinities outside of tolerated ranges are known to cause osmotic stress and impact development in fishes, but experimental data are limited among estuarine fishes. Estuaries are also vulnerable to contamination by per- and polyfluoroalkyl substances (PFAS), a large class of manmade organoflourine compounds that are used in a variety of industrial and commercial applications, due to the proximity to industrialized areas. These compounds are highly mobile, persistent, and ubiquitously detected in sediment, soil, surface waters, wildlife, and humans. Due to the broad range of applications for PFAS, these chemicals are in constant production, which contributes to their continuous release into the environment via point and non-point sources. However, it is known that one of the primary sources of PFAS in the environment are aqueous film-forming foams (AFFFs), which are used by many industrial sites to extinguish hydrocarbon-based fires. Despite estuarine habitats along the Gulf of Mexico being particularly vulnerable to PFAS contamination due to their proximity to sites utilizing AFFF and significant natural salinity fluctuations, toxicological studies examining the effects of PFAS on aquatic biota overwhelmingly utilize freshwater models. Therefore, the present study aims to address important data gaps regarding the separate and combined effects of PFAS contamination and hypersalinity. Early life stage red drum (*Sciaenops ocellatus*) were selected for this study due to their economic and ecological importance, as well as their abundance in the Gulf of Mexico, a system which experiences both fluctuations in salinity and contamination by PFAS. Perfluorohexanesulfonic (PFHxS) was chosen as the PFAS of interest as it is a major component of AFFF. These data have implications for the survival of early life stage larval red drum residing within estuaries that have the potential to encounter both hypersaline conditions and PFAS.

**3.04.P-Th066 Consideration of Site-Specific Conditions and Food Web Structures in Per- and Polyfluoroalkyl Substances (PFAS) Bioaccumulation: A Watershed-Scale Case Study**

***Sagar Thakali****, Christine Archer, Heather Loso, Maryann Welsch and Betsy Ruffle, AECOM*

Bioaccumulation of per- and polyfluoroalkyl substances (PFAS) is a topic of critical importance in understanding their potential environmental impacts. Factors contributing to wide ranging variability in PFAS bioaccumulation in fish and other biota include the nature and extent of PFAS releases, environmental conditions, and the characteristics of food webs, all of which are site-specific. Approaches to evaluating these site-specific factors holistically at a given site is critical in understanding the observed variabilities in PFAS bioaccumulation.

To support the 2021 Baseline Ecological Risk Assessment that AECOM performed, extensive sampling of abiotic (surface water, sediment, pore water, and foam) and biotic media (aquatic plants, crayfish, snails, amphibians, forage fish, and predatory fish). was conducted in the waterways associated with Project 1007 (Minnesota, USA). Altogether 41 PFAS were measured, including short and long-chain carboxylates and sulfonates, fluorotelomers, and sulfonamides.

PFAS distributions in abiotic media reflected the nature of the releases, compound-specific physicochemical differences owing to different chemical structures, and regional hydrogeology and geochemistry. Perfluorooctane sulfonic acid (PFOS) dominated the distribution and speciation in abiotic media, followed by perfluorooctanoic acid (PFOA), perfluorohexane sulfonic acid (PFHxS), perfluorobutanoic acid (PFBA), and other shorter chain PFAS. PFOS also dominated the distribution in biotic media. PFOS concentrations were higher in predatory than foraging fish and lowest in snails and crayfish, reflecting trophic level and food web structure. Distribution of other PFAS in biota varied in different organisms, e.g., after PFOS, perfluorooctane sulfonamide (PFOSA) dominated in crayfish. Among aquatic plants, floating plants (e.g., duckweed) had the highest PFAS, reflecting differences in the exposure media, uptake and accumulation mechanisms. Based on on-going evaluation of the data, important factors and interactions that contribute to PFAS bioaccumulation at various trophic levels will be identified.

The overall objective of the presentation is to provide an approach that can be extrapolated to different sites for a holistic consideration of critical site-specific factors in evaluating PFAS bioaccumulation. We will present data from a watershed-scale case study and conceptual site model(s) to elucidate the site-specific and general findings.

**3.04.P-Th067 PFAS Trends in Freshwater Fishes of New York State**

***Eric Levanduski**[1], Wayne Richter[2], Jesse Becker[2], Yasaman Hassanzadeh[2] and N. Roxanna Razavi[1], (1) SUNY College of Environmental Science and Forestry, (2) New York State Department of Environmental Conservation*

Per- and poly-fluoroalkyl substances (PFAS) are an extremely large (> 4,700) group of fluorinated chemicals that exist in many industrial and consumer products and have been widely used as firefighting agents (e.g., aqueous film forming foams, AFFF) at airports and defense sites. Currently there is widespread concern from the public regarding the human and environmental health impacts of these “forever chemicals”. There is an urgent need to characterize PFAS contamination that is now ubiquitous across the nation’s waters. Here, we present a plan to mine and analyze the New York State Department of Environmental Conservation (NYSDEC) dataset of New York State PFAS fish concentrations to answer specific questions such as species-specific bioaccumulation patterns, potential for biomagnification, and geographical trends across New York State freshwaters. Using fishes collected between 2016-2021 (n = 2941), we present a statistical design to ask how PFAS accumulate in different fish taxa and by feeding habitat and assess trophic level relationships among fish species as predictors of PFAS profiles. To evaluate geographical trends, we describe a statistical approach to compare PFAS concentrations in fish from Great Lakes waters (including the Niagara and St. Lawrence Rivers) to background concentrations in inland waters. We anticipate this project will provide an improved characterization of PFAS concentrations in fish that will be used to effectively set fish consumption advisories, enable NYSDEC to apply fish concentrations to both ecological and human health risk assessment, and provide

a better understanding of sampling considerations including species and locations of concern for future PFAS investigations by NYSDEC and the larger scientific community.

**3.04.P-Th069 Body Compartment Partitioning and Ecological Effects of PFAS Mixtures in a Multi-Species System**

*Lindsay Holden[1], **Allison Narizzano**[1], Andrew East[1] and Natalie K. Karouna-Renier[2], (1) U.S. Army Public Health Center, (2) U.S. Geological Survey*

Little is known about the impacts of exposure to per- and polyfluoroalkyl substance (PFAS) mixtures, but ubiquitous contamination of soil and water at and around military installations with current and historical use of PFAS-containing aqueous film forming foams (AFFFs) is an environmental and health safety concern. In this project, we will assess how exposure to individual PFAS and PFAS mixtures partition into different biological compartments, travel up the food chain, and affect development in keystone species. The specific objectives of this project are to quantify the dynamics of whole body burden and serum concentrations of individual PFAS and PFAS mixtures in mice and the effects of PFAS trophic transfer in the American kestrel, an avian species that consumes rodents and can be found on military sites throughout the country. *Mus musculus* will be exposed via oral gavage to individual PFAS or PFAS mixtures and assessed for whole body burden and serum PFAS concentrations. Results for the mixtures will be compared to the individual PFAS exposure results for identification of mixture influence on PFAS retention and/or elimination patterns. American kestrel pairs will be dosed with a PFAS mixture in their diet at a concentration based on PFAS whole body burden data collected in *Mus*, and allowed to mate and lay eggs. A subset of eggs will be assessed to evaluate PFAS transfer. The remaining eggs will be naturally incubated to determine effects on hatch rate. Hatchlings will be orally dosed with the PFAS mixture and allowed to fledge. Fledgling serum and tissue samples will be analyzed for PFAS concentrations and plasma will be analyzed for immune and thyroid indicators. Exposure-dependent effects observed in mice, eggs, and fledglings will be modeled at their individual trophic levels and within an ecosystem interaction model to understand potential population-level effects. These data are needed for environmental remediation prioritization and ecological risk assessments and will establish a framework for understanding if PFAS mixtures behave differently than individual PFAS. This project will collect data relevant to One Health approaches to chemical management on military lands and specifically address the need to understand the toxicity of PFAS mixtures released from AFFFs into the environment.

**3.04.P-Th070 Thyroid Hormone Disruption Potentials of Two Fluorotelomers, 6:2 Fluorotelomer Sulfonate and 6:2 Fluorotelomer Alcohol in Embryo-Larval Zebrafish (Danio rerio)**

***Eun-Jin Kim**, Ba Reum Kwon, Ah-Reum Jo, Yeonju Nam, Gowoon Lee and Kyungho Choi, Seoul National University, Korea, Republic of (South)*

Due to unique water and oil repelling properties, per- and polyfuoroalkyl substances (PFASs) have been widely used in various products such as cooking utensils, textiles, cosmetics, and fire-fighting foam. As toxicities of some of traditional PFASs have been recognized, alternatives such as 6:2 fluorotelomer sulfonate (6:2 FTS) and 6:2 fluorotelomer alcohol (6:2 FTOH) have been increasingly used and hence frequently detected in water environment. However, little is known about the thyroid disrupting effects in aquatic organisms. We investigated adverse effects of 6:2 FTS and 6:2 FTOH in thyroid system and possible mechanisms using embryo-larval zebrafish. Zebrafish embryos were exposed to 0.3~10 µM 6:2 FTS and FTOH for 120 hours, and measured for embryo and larval survival, hatchability, malformation rate. In addition, thyroid hormones (TSH, T4, T3) and the transcription of the related genes were measured. Exposure to 6:2 FTS and 6:2 FTOH significantly influenced the time-to-hatch and malformation rate during embryo-larval period. In addition, significant changes in whole body TH levels were observed along with regulatory changes of genes in the HPT-axis. Following exposure to 6:2 FTS, the levels of all measured thyroid hormones were observed. Up-regulation of the genes related to thyroid hormone synthesis, e.g., *trh*, *tshb*, *nis*, *tpo*, or *tg* genes, or down-regulation of a gene related to the excretion, *ugt1ab* gene were generally observed. For 6:2 FTOH, however, TSH and T3 were decreased, and the transcription of *trh*, *tshb*, *tpo*, or *tg* genes were also reduced. The genes related to elimination of the hormones, e.g., *ugt1ab* or *sult1st5* were up-regulated. Overall, our results demonstrate that two fluorotelomers have a potential for thyroid disruption by interfering central regulation, synthesis and metabolism of thyroid hormones, however in different directions. Further studies are needed to confirm different mode of action of thyroid disruption and related health consequences in other animal models or stages of life.

**3.04.P-Th071 Exploring Toxicokinetics of PFAS Across Exposure Routes, Life Stages, and Sex Across Taxonomically Diverse Amphibians**

***Meredith N. Scherer***, *Tyler D. Hoskins, Connor M. Neill, Lucy E. Burcham, Sophia R. Horn, Youn Jeong Choi, Linda S. Lee, Jason T. Hoverman and Marisol S. Sepulveda, Purdue University*

Per- and polyfluoroalkyl substances (PFAS) are ubiquitous environmental contaminants that pose hazards to human and wildlife health. While PFAS kinetics are well-studied in mammals and fish, less is known for amphibians. Amphibians are an excellent model because of their complex life histories: aquatic larvae metamorphose into air-breathing metamorphs. Therefore, amphibians provide an opportunity to test kinetics across important routes (dietary, water, dermal) and life stages (aquatic larvae, terrestrial adults, or aquatic adults). We previously conducted experiments with structurally diverse PFAS (PFOS, PFOA, PFHxS, and 6:2 FTS) to describe bioconcentration factors (BCFs), uptake, and elimination across multiple species, routes and life stages. Despite knowledge gained from studies with Indiana native frogs, toads, and salamanders, toxicokinetic studies are lacking for the amphibian model *Xenopus laevis*, which differs from native species taxonomically and by life history. Further, the possibility of sex-biased elimination is unexplored as well as whether elimination rates are reduced after metamorphosis, despite that loss of gills suggests this possibility. We present a summary of past work on general patterns in PFAS kinetics among native amphibians along with results from two new experiments that quantify BCFs and capture rapid uptake curves in *X. laevis* larvae. We hypothesize that 1) sexes differ in elimination rates, and 2) that elimination slows after metamorphosis. In an uptake experiment, we exposed *X. laevis* larvae to 0, 10, or 1000 mg/L of PFOS, PFOA, and PFHxS and sampled heavily early during exposure (2.5, 5, 24, 48, 120, 168, and 240 h) to quantify rapid uptake and steady-state BCFs. In a depuration experiment, we exposed *X. laevis* larvae to PFOA at 1.2, 3.7, 11.1, 33.3, and 100 mg/L and measured body burdens and elimination rates for larvae and metamorphs. Larvae were exposed for 14 days before depuration and sampled at 0, 10, and 20 d, and Nieuwkoop Faber stages 62 and 66 to determine larval steady-state BCFs and depuration rates. Larvae were exposed through metamorphosis and sampled at 0, 10, 20, 30, and 40 d to determine post-metamorphic steady-state BCFs and depuration rates. All animals were genotypically sexed. Results increase our understanding of toxicokinetics across species, PFAS, life stages, exposure routes, and sexes among amphibians, which are exposed in the field and differ from fish and mammals in PFAS kinetics.

**3.04C PFAS and Related Compounds in Terrestrial and Aquatic Wildlife: Exposure, Uptake, Tissue Distribution, and Toxic Effects**

**3.04C.T-01 Introductory Remarks - PFAS and related compounds in terrestrial and aquatic wildlife: exposure, uptake, tissue distribution, and toxic effects**

***Natalie K. Karouna-Renier***[1], *Cheryl A. Murphy*[2], *Christine M. Custer*[1] *and Michael W. Murray*[3], *(1) U.S. Geological Survey, (2) Michigan State University, (3) National Wildlife Federation, United States*

**3.04C.T-02 Measurement of the Solubility of Serum Albumin for Perfluorooctane Sulfonate (PFOS) via Rapid Thin-Film Solid Phase-Microextraction**

***Sophia Shu Han Hsu***, *Barry C. Kelly and Frank Gobas, Simon Fraser University, Canada*

Per- and Polyfluoroalkyl Substances (PFAS) are persistent, bioaccumulative chemicals that are ubiquitous in the environment, wildlife, and humans. Unlike other contaminants, PFAS associate strongly with protein- and phospholipid-rich tissues. It is thus imperative to have reliable solubility and partitioning properties for PFAS to better evaluate their bioaccumulative potential and toxicity. In particular, the solubility or sorptive capacity can be used to calculate the chemical activity ($a$), a unitless concept increasingly applied in chemical risk assessments for direct comparisons of toxicity data of different medium types. The chemical activity is determined with the ratio of concentration ($C$) and solubility ($S$) for liquids (i.e., $a = C / S$), and with the adjustment of the fugacity ratio ($F$) for solids (i.e., $a = C \times F / S$). Essentially, knowing the solubility of biological media for PFAS enables the activity-based assessment of bioaccumulation, where data of different phases can then be integrated and analyzed. In this study, a method using rapid thin-film solid phase-microextraction (TF-SPME) via ethylene vinyl acetate is developed to derive the solubility and partitioning coefficient for biological media. Specifically, the solubility of bovine serum albumin ($S_{BSA}$) for perfluorooctane sulfonate (PFOS) is tested for preliminary optimization. Results show that $S_{BSA}$ ranges from 32 to 58 g/L and that Log BSA-water partition coefficient ($K_{BSA\text{-}water}$) ranges from 2.7 to 3.3 for PFOS exposure from 23 to 140 ng/mL. The TF-SPME equilibrates in 15 minutes and does not deplete PFOS from the partitioning medium, making it a reliable and cost-efficient method. The results in this study lay groundwork for finding the solubility of other biological media for PFOS as well as other PFAS, thereby facilitating activity-based PFAS risk assessment.

**3.04C.T-03 PFAS in Wildlife: Lessons from 15 years of Occurrence Data and New Standard Methods including EPA 1633**

***Bharat Chandramouli***, *Coreen Hamilton and Million Woudneh, SGS AXYS Analytical Services Ltd., Canada*

PFAS are persistent as a chemical class and specific PFAS are bioaccumulative with aquatic exposure levels for humans in the low parts per trillion levels. Two decades worth of PFAS monitoring in biota has focused on a short list of perfluoroalkyl carboxylates (PFCA) and sulfonates (PFCA) such as PFOA and PFOS. Given the phaseout of long chain PFAS, and perfluorinated alkyl acid chemistries and their replacement with shorter chain, telomer, and per/polyfluoroether equivalents, there is an urgent need to understand the occurrence and fate of these other PFAS. In this study, we developed and validated isotope dilution methods for an expanded suite of 40 diverse PFAS in aqueous, solid, tissue and serum matrices. This internal method formed the basis for the new EPA standard method 1633. We applied these methods to multiple ecosystem compartments including water, soil/sediments and fish in North America. PFAS monitored included C4-C14 perfluoroalkyl carboxylates, perfluoroalkyl sulfonates C4-C10, C12, fluorotelomer alkyl acids 4:2, 6:2, 8:2 FTS and 3:3, 5:3 and 7:3 FTCA, ether PFAS HFPO-DA (GenX), ADONA, F53-B, PFMBA, PFMPA and PFEESA and C8 sulfonamide precursors/intermediates including FOSA, MeFOSA and EtFOSA, MeFOSAA and EtFOSAA, and MeFOSE and EtFOSE. Occurrence data in animal tissue from this expanded method was combined with legacy data from 2006 onwards on methods that used the same isotope dilution/surrogate standard quantification approaches, providing continuity of measurement approach. We will present occurrence and aggregate estimates of concentration data spanning 15+ years of PFAS analysis. Preliminary results indicate that even with phaseout, PFOS is still the predominant PFAS in tissues. The use of extended monitoring lists show significant and enduring presence of long-chain PFAS such as C13, C14 PFCA due to their bioaccumulative properties. We will also discuss how best practices on tissue PFAS measurement have evolved and eventually coalesced into EPA 1633 draft.

**3.04C.T-04 Getting From Here to There: Testing the Applicability of Bioaccumulation Factors for PFAS for Freshwater Fish**

***Kenia Whitehead***, *James K. Wilhelm, Evelyn Reategui Zirena and Philip E. Goodrum, GSI Environmental, Inc.*

As noted by U.S. EPA in the draft aquatic life ambient water quality criteria for two per- and polyfluoroalkyl substances (PFAS) in May, bioaccumulation potential is a key factor in the development of protective risk-based criteria. The diversity of PFAS chemical characteristics combined with varying environmental factors has created a unique set of challenges in developing predictive models of bioaccumulation potential in different organisms. Field derived bioaccumulation factors show significant variability, typically ranging several orders of magnitude. The current study focuses on PFAS accumulation into freshwater fish and the use of bioaccumulation factors from surface water (BAF) and/or sediment (BSAF) calculated from field data collected from various locations in the northeastern United States. Fish data include various trout, perch, bluegill, and bass species. Bivariate and multivariate analyses were conducted on a wide range of factors including PFAS physio-chemical parameters (e.g., chain length, functional group), fish metrics and ecology (e.g., fish age, length/weight, feeding mode, trophic level), and environmental factors ((e.g., total organic carbon in sediment and surface water). We present results for these sites that show: 1) the potential utility of data standardization in BAF/BSAF formulation; 2) how ratio-based BAF/BSAF estimates compare with more general regression models; and 3) emerging patterns in the set of chemical properties and environmental factors that contribute to variability in concentrations in whole body fish. Given the complexity of chemical transport and feeding behavior of the fish across trophic levels, PFAS may not be amenable to a single approach or set of statistical criteria to derive predictive bioaccumulation models. These results indicate that PFAS uptake in fish is mediated via direct and indirect pathways from sediment and surface water to organisms. Our analysis further highlights the complex pathways of PFAS from abiotic media into fish and indicates that caution should be applied in the use of BAF/BSAF ratios to predict fish tissue concentrations from surface water and sediment.

**3.04C.T-05 Using a Probabilistic Approach in an Ecological Risk Assessment PFAS Food-Web Based Model**

***Katherine Parakal***[1]*, Jason M. Conder*[2] *and Roman Lanno*[1]*, (1) The Ohio State University, (2) Geosyntec Consultants, Inc.*

The determination of PFAS bioaccumulation is crucial to understanding hazards associated with PFAS at contaminated sites, however, tissue sample analysis can be complicated and expensive. As a result, models that link toxicity, bioaccumulation, and trophic transfer are needed. In the past year, a food-web based model was developed that allows the user to use measured concentrations of PFAS in abiotic media to estimate PFAS concentrations at different trophic levels in aquatic food webs. While the model provides a useful tool for risk estimation, it takes a deterministic approach. However, probabilistic risk assessment provides a means for characterizing uncertainty and variability, enabling decision makers to assess uncertainty in relation to environmental management decisions. Thus, the goal of this research is to further develop the model into a probabilistic model. In order to achieve this, the standard deviations of PFAS uptake factors were incorporated into the model from available literature. Studies that lacked this data were examined for standard errors, coefficients of variance, PFAS uptake, and elimination rate constants to compute standard deviations. Following that, Crystal Ball was enabled in the model that allows the user to run several simulations and obtain PFAS tissue concentration distributions at different trophic levels. These probability distributions are crucial for understanding the uncertainty associated with risk estimates. Moreover, the model can be used to identify the key drivers of variability in PFAS tissue predictions. Median perfluorooctanoic acid(PFOA) concentration values in sediment and surface water from available literature were used in the model to determine the main sources of variability in PFOA tissue predictions. The results showed that the major source of variability for PFOA in fish came from biomagnification factor of fish (BMF-fish). For mammals, the main source of variability came from bioconcentration factor (BCF) in pelagic invertebrates (77%) and a smaller portion (23%) from BMF-fish, BCF-fish, and biota sediment accumulation factor. Thus, enabling probabilistic capacity and running the Monte Carlo simulations can aid in determining where the major data gaps lie and can direct future research efforts. This model enhancement will allow a better characterization of risk, communication of uncertainty around risk estimates, and contribute to a better understanding of mechanisms that affect PFAS bioaccumulation.

**3.04C.T-06 Validation of Food Web Models for Per- and Polyfluoroalkyl Substances or PFAS**

***Jean Zodrow***[1]*, Jason M. Conder*[1]*, Marianne Batchelder*[1]*, Caitlin Johnson*[1] *and Katherine Parakal*[2]*, (1) Geosyntec Consultants, Inc., (2) The Ohio State University*

Food web models allow ecological risk assessors to use concentrations of per- and polyfluoroalkyl substances (PFAS) in abiotic media (soil, sediment, water) to predict concentrations of PFAS in wildlife diet items, enabling estimation of site-specific dietary doses for wildlife, a critical part of ecological risk assessments (ERAs) at PFAS-impacted sites. While food web models provide a useful tool for ERA, there are concerns that food web models for PFAS have not met the level of validation obtained by food web models routinely used for ERAs of other chemicals (e.g., polychlorinated biphenyls, metals, pesticides). This concern can lead to a lack of confidence around food web modeling for PFAS which prevents utilizing food web models at PFAS-impacted sites. Abiotic media concentrations and other data important for food web modeling have emerged at many PFAS sites in the last few years since food web models for PFAS were first developed, allowing a robust validation of these models. Data from these field studies, including abiotic media such as surface water and sediment at aquatic sites and soil at terrestrial sites, can be used to generate predictions of PFAS in tissue (using food web models) that can be compared to the measurements of PFAS in biota (invertebrates, fish, plants). In this way, the food web model accuracy can be assessed, providing an important validation of the models for use in site-specific ERAs. In this study, several aquatic and terrestrial PFAS-impacted sites with high quality data were selected for validation. Model-predicted concentrations of PFAS (primarily perfluoroalkyl carboxylates and sulfonates) in biota (i.e., invertebrates, plants, and fish) were generally within a factor of 5 of the measured values. Importantly, the model predictions generally agreed with measurements in terms of identifying the biota with the highest concentrations of PFAS at the sites. This validation effort provides confidence in PFAS food web modeling and helps identify areas of continued uncertainty and refinement of modeling efforts.

**3.04C.T-07 Comparing activity and concentration-based trophic magnification factors of perfluoroalkyl substances within an urban terrestrial food-web**

***Kate Fremlin***[1], *John E. Elliott*[2], *Robert J. Letcher*[2], *Tom Harner*[2] *and Frank Gobas*[1], *(1) Simon Fraser University, Canada, (2) Environment and Climate Change Canada, Canada*

Improving understanding of the biomagnification potential of perfluoroalkyl substances (PFAS) within terrestrial organisms and food-webs is essential for establishing effective guidelines that protect air-breathing organisms. Ionizable surfactants like PFAS often do not biomagnify in water-breathing organisms due to their high aqueous solubility and low volatility. However, these characteristics may result in limited respiratory elimination of PFAS in air-breathing organisms and contribute to biomagnification. Also, PFAS do not typically accumulate in neutral lipids, so methods commonly used to estimate trophic magnification of legacy organic chemicals may not be applicable. Thus, there is a need to develop suitable approaches to assess bioaccumulation of PFAS in air-breathing organisms. Accordingly, we assessed biomagnification of a suite of PFAS in a terrestrial food-web that included poikilothermic and endothermic organisms. Each biotic sample was evaluated for total protein, albumin, structural protein, and polar lipid content to account for differences in preferential sorbing matrices between organisms. We compared Trophic Magnification Factors (TMFs) for PFAS using three approaches: 1) wet weight concentrations ($TMF_W$); 2) tissue normalized concentrations (e.g., total protein ($TMF_P$); polar lipids ($TMF_{PL}$); albumin and polar lipids ($TMF_{PPL}$); albumin, polar lipids, and structural protein ($TMF_N$), and 3) chemical activities ($TMF_A$). All TMFs for PFNA, PFDA, PFOS, and PFDS were statistically > 1 indicating consistent biomagnification while all TMFs for PFBS were statistically < 1 indicating consistent dilution in this food-web. PFUdA, PFDoA, PFTrDA, and PFTeDA had $TMF_{PL}$s ≤ 1 while all other TMFs were statistically > 1. PFOA, PFHxDA, and PFHxS only had $TMF_W$s statistically > 1 while all other TMFs were ≤ 1. $TMF_A$s for most PFAS were equivalent to the other associated TMFs demonstrating that activity is an analogous method to use for TMF determination.

**3.04C.T-08 Tissue Distribution of PFAS in Avian Species: Uncertainties and Data gaps**

***John L. Newsted***, *Ramboll*

Internal concentrations of chemicals have been used in ecological risk assessments as a more realistic measure of exposure in that it takes into account aspects of bioavailability, routes of exposure, assimilation, and metabolic capacity of an organism. Tissue-based toxicity reference values (TRV) for organisms such as birds, typically consists of extrapolating toxicity data from a surrogate species to a species of interest. Furthermore, many TRVs have been derived for persistent and lipophilic organic chemicals where tissue accumulation and distribution are influenced lipid content. Perfluoroalkyl substances (PFAS) represent a large, diverse group of chemicals that with varying capacities, partition into lipid as well as also bind to various proteins. As a result, the tissue distribution of PFAS can vary based on the chemical structure including chain length and type of terminal group. Other factors including inter- and intraspecies-specific differences in physiological and biochemical processes as well as changes in environmental conditions including temperature and the presence of other chemical classes may also alter tissue distribution. To address this issue, a critical examination of tissue distribution data from laboratory and field studies was conducted with avian species. Aspects related to species and tissue specific differences in metabolic capacity and protein binding characteristics along chemical structure related to fluorocarbon chain length, functional head group will be evaluated and discussed. Insights into how this information can be used to establish TRVs in birds will be discussed along with the types of studies that still need to be conducted to better reduce the uncertainties related to tissue distribution of PFAS.

## 3.05 Wildlife Risk Assessment in the 21st Century: Integrating Emerging Science in Advancing Risk Assessment for Wildlife

### 3.05.T-01 Using Exposomics to Characterize Effects of Environmental Chemical Exposure in Wildlife

***Mary Ann Ottinger***[1] *and Cullen Geiselman*[2]*, (1) University of Houston, (2) The Cullen Trust for Health Care*

Avian populations have steeply declined over the past century, associated with anthropogenic factors originating from industry, urbanization, changing land use, habitat loss, pollution, emerging diseases, and climate change. All combine to exert increasing stress and impair health with diminished metabolic, immune, and reproductive function, deteriorating overall health, and reduced longevity. The health of avian populations, ecosystem health, and human health are inexorably linked, and wildlife conservation is fundamental to sustaining healthy ecosystems. This interrelationship is captured in the One Health concept; however, the precise measures of exposure and effects often are not well characterized for individuals. The Exposome provides another conceptual framework, which is focused on the effects of environmental stressors and chemical exposures at specific life stages and throughout the lifespan of an individual. Effects are assessed using physiological end points (exposomics) that reflect a range of molecular mechanisms, physiological and behavioral responses. The Exposome concept has focused on humans and has not been fully developed for wildlife. Developing an exposomic approach that incorporates reliable metrics of exposure to environmental chemicals and other stressors can be a powerful approach, especially when integrated into a One Health framework for assessing risk and developing mitigations. Exposomics will also provide essential information for managers of protected lands and for programs aimed at early prediction of disease and environmental chemical exposures in wildlife. We discuss these conceptual frameworks from the perspective of the human-wildlife-ecosystem health nexus and for developing proactive approaches to conserving avian populations and other wildlife.

### 3.05.T-02 Suggestions for Improving Risk Assessments for Terrestrial Wildlife: Outcome of a Workshop

***Bradley E. Sample***[1]*, Mark S. Johnson*[2]*, Thomas Bean*[3] *and Nico van den Brink*[4]*, (1) Ecological Risk, Inc., (2) U.S. Army Public Health Center, (3) FMC Corporation, (4) Wageningen University & Research, Netherlands*

Wildlife risk assessment is the use of data and analytical tools to support informed environmental management decisions concerning chemical hazards to wildlife. This process has changed little in over thirty years despite significant advancements in our understanding of estimating exposure and effects. A SETAC Workshop on ‘Wildlife Risk Assessment in the 21st Century: Integrating Advancements in Ecology, Toxicology, and Conservation’ provided an opportunity for ecologists, biologists, risk assessors, and exposure scientists to discuss these advancements and make useful recommendations. Here we provide a summary of the outcome of this workshop and propose opportunities to advance risk assessments for terrestrial wildlife species.

### 3.05.T-03 Key Challenges and Developments in Wildlife Risk Assessment Problem Formulation

***Bradley E. Sample***[1]*, Kurt A. Gust*[2]*, Ruth Hull*[3]*, Lawrence Kapustka*[4]*, Wayne G. Landis*[5]*, Jan-Dieter Ludwigs*[6]*,*

*Gary Mann[7], David Mayfield[8], Wayne Munns[9], Cheryl A. Murphy[10] and Mary Sorenson[11], (1) Ecological Risk, Inc., (2) U.S. Army Engineer Research and Development Center, (3) Gary D. Williams & Associates Inc., Canada, (4) LK Consultancy, Canada, (5) Western Washington University, (6) RIFCON GmbH, Germany, (7) Azimuth Consulting Group Inc., Canada, (8) Labcorp, (9) U.S. Environmental Protection Agency, (10) Michigan State University, (11) ENVIRON International Corporation*
Problem formulation is a critical step in planning a risk assessment for chemical exposures to wildlife. The value of the risk assessment depends on how well it meets the goals and specifications defined through the problem formulation process, and how it facilitates subsequent decision making. Extensive regulations, policies, and guidance exist for conducting wildlife risk assessment, and although some have been recently updated, the methods to evaluate risks to wildlife have not markedly changed in decades. Meanwhile, our understanding of exposure modifying factors, toxicology, and the underlying species-specific wildlife ecology has expanded considerably. Novel methods, approaches, and techniques continue to emerge that have application to wildlife risk assessment and hold promise to increase accuracy in risk predictions providing ecological relevance and this needs to be better linked directly to decision-making. Similarly, some existing approaches can also be applied in novel ways with similar benefits. Evaluating opportunities to incorporate such advancements into wildlife risk assessment should be an ongoing effort. As part of a SETAC workshop initiated to evaluate the potential of emerging science to improve wildlife risk assessment, we summarize multiple challenges to defining the problem in conducting chemical risk assessments for wildlife, along with potential developments to address these challenges. These challenges and developments include: (1) clearly identifying protection goals linked to possible management actions, while acknowledging any constraints; (2) collecting sufficient relevant information to answer the question under investigation to allow for an informed decision; (3) understanding ecological influences of chemical exposures in a multi-stressor environment; (4) including consideration of relative influences of biological and physical stressors explicitly, putting chemical stressor risk in ecological context; (5) adopting ecological theory and practices in defining assessment endpoints; (6) increasing the focus on functional ecological processes at the population level; (7) using Bayesian networks, advances in handling big data and data from disparate streams (e.g., integrating Adverse Outcome Pathways, eDNA or sRNA), and other such tools when the scope of the risk assessment would benefit from the added complexity, increasing transparency in assumptions including those related to stochasticity or population modelling.

### 3.05.T-04 Entrenchment of risk assessment practices that thwart advancement

***Lawrence Kapustka**, LK Consultancy, Canada*
The challenges in quantifying risk to ecological receptors are many. As I noted in an address in 2006 and published in 2008 (IEAM 4:209-298), some of the limitations are inherent, but others are contrived. In the16 years since I formulated the lists, ironically more has been done to address the inherent limitations that the contrived ones. Developments in computing capacity to deal with big data, to perform complex modelling routines, including exploration of probability of alternative outcomes provide deeper understanding of stochasticity and causality in various scenarios. Refinement and speed of methods to detect and map environmental DNA and functionality through sRNA further advance our collective ability to interpret ecological processes. Similarly, the adoption of landscape perspectives and recognition of the importance of thinking in terms of complex social-ecological systems provides a better foundation for engaging in meaningful dialogue regarding management options to identify and deal with risks. Unfortunately, while these technical advances have occurred, there is stagnation within the applications of ecological risk assessment. There continues to be too many cases in which NOAECs and LOAECs are employed. Risk quotients are still being used despite their inherent limitations (non-scalable) and frequently, even added to project cumulative risk. And further, risks posed by individual chemicals are seldom considered in context of other stresses including habitat quality and landuse practices that may be more consequential. I will revisit the short-term and long-term actions that I first proposed in 2006 and offer suggestions going forward.

## 3.05.T-05 Revision of the EFSA Guidance Document on Birds and Mammals: Industry view

***Thomas Bean***[1], *Alex Blakey*[2], *Markus Ebeling*[3], *Manousos Foudoulakis*[4], *Paula Garcia*[5], *Sonja Haaf*[6], *Joerg Hahne*[3], *Steven Kragten*[7], *Judith Neuwohner*[8], *Joachim Nopper*[9], *Dennis Sprenger*[9] *and Arnd Weyers*[3], *(1) FMC Corporation, (2) Syngenta, United Kingdom, (3) Bayer CropScience LLC, Germany, (4) Corteva Agriscience, Greece, (5) Corteva Agriscience, United Kingdom, (6) ADAMA Deutschland GmbH, Germany, (7) Syngenta Ltd, Germany, (8) Nufarm Europe GmbH, Germany, (9) BASF SE, Germany*

The European Food Safety Authority (EFSA) Guidance Document on the Risk Assessment for Birds and Mammals [EFSA, 2009)] (EFSA GD) has been used for more than 12 years in the EU for the evaluation of Active Substances and Plant Protections Products. As countries in other regions of the world look to the EU when evaluating registrations, any changes to the EFSA GD have the potential for global impact. Recently, EFSA has launched the public consultation on the draft updated guidance document on Risk Assessment for Birds and Mammals [EFSA, 2021)].

The risk assessment scheme of the current EFSA GD has proven generally adequate to evaluate the acute risks to birds and mammals. The scheme for refined risk assessments, particularly for the reproductive risk, has had major issues. Numerous changes have been proposed in the new draft EFSA GD, however its impact on risk assessment cannot be fully evaluated as key aspects are unavailable e.g., EU protection goals are still pending and the updated Tier 1 calculator tool has not been provided.

Regulatory scientists and risk assessors working for member companies of Crop Life Europe (CLE) use this Guidance in their daily work. Their expert view of where the updated draft could be improved has been gathered, submitted and is shared here. Contributing with specific expertise as for this occasion, industry have mobilized not only the Crop Life Europe Expert Group on Terrestrial Vertebrates but also the CLE Expert Groups on Statistics, Effect Modelling, Residue Definition, Toxicology and environmental fate.

In this presentation, a major focus will be on advances (e.g., new residue unit dose [RUD] values) and shortcomings of the draft GD in the area of harmonization and to highlight topics that require further revision as the provided explanations are not conclusive and/or their implementation is not practical.

Examples include: Benchmark Dose approach and the selection of the ecotoxicologically relevant reproductive endpoint, the use of the $f_{TWA}$ and agreed Tier 1 risk assessment, and further clarity or discussions on the higher tier options and modelling. These parameters will be described in more detail in the platform presentation.

To minimise contradictory interpretations by risk assessors and to maximise the development of a clear, acceptable GD, these CLE experts would support engaging further with the GD authors to resolve these discussion points to build a path forward to a successful update guidance.

## 3.05.T-06 Use of Population Modeling in Wildlife Risk Assessments

***Carolyn Meyer***, *Holly McChesney, Timothy Walker, Emily Morrison and Alex Francisco, Arcadis U.S., Inc.*

Population modeling is a tool receiving increasingly greater use in wildlife ecological risk assessments. We discuss why population modeling should be used and contrast population modeling approaches with standard risk assessment approaches. We show how such models can be structured to create a more holistic understanding of wildlife risk, and describe the key endpoints to evaluate, which include population size, growth rate, and probability of extinction or quasi-extinction. We describe different types of models or components in the models that can be included, and the benefits and applications of each, particularly how the models might be used if toxicity testing using animals is reduced in the future. We demonstrate the approach with various examples and case studies. Most importantly, because interpretation of the output of population models can be variable depending on the investigator, we suggest some approaches to standardizing the interpretation of risk based on the output of such models.

**3.05.T-07 Development and application of a generic avian physiologically-based kinetic (PBK) model for three bird species**

*Vanessa Baier[1], Alicia Paini[1], Stephan Schaller[1], Colin G Scanes[2], Markus Ebeling[3], Thomas Preuss[3], Johannes Witt[3], David Heckmann[3] and* ***Audrey Bone****[3], (1) esqLABS GmbH, Germany, (2) University of Arkansas, (3) Bayer CropScience LLC, Germany*

Physiologically-based kinetic (PBK) models are powerful tools for designing toxicological studies and conducting extrapolations. These extrapolations are done to inform hazard characterization in risk assessment by filling data gaps and defining safe levels of chemicals. In the present work, an avian PBK model for male and female birds was developed using PK-Sim and MoBi from the Open Systems Pharmacology Suite (OSPS). The PBK model includes an ovulation model to predict concentrations of chemicals in eggs from dietary exposure. In order to correctly represent chickens (*Gallus gallus*), bobwhite quails (*Colinus virginianus*), and mallard ducks (*Anas platyrhynchos*) within the generic PBK model, a comprehensive systematic review and analysis were conducted on the physiology of these species. After integration of the avian physiologies into the PBK models, the models were tested for nine chemicals for which *in vivo* studies were available. Time-concentration profiles of chemicals reaching tissues and egg compartments were simulated and compared to *in vivo* data by assessing the 3- to 10-fold deviation. The overall accuracy of the PBK model predictions across the analyzed chemicals was good. However, for some compounds scarcity of in-vivo data and inconsistencies between published studies allowed only limited goodness of fit evaluation. The generic avian PBK model was developed following a "best practice" workflow on how to build a PBK model for novel species. The credibility of the avian PBK models was scored by evaluation according to the available guidance documents from WHO and OECD to increase applicability, confidence, and acceptance of these *in silico* models in chemical risk assessment .

**3.05.T-08 Geospatial Risk Assessment Using High-Throughput Screening Assays to Quantify Potential Adverse Effects From Exposure To Chemical Mixtures**

***Kristin M. Eccles****, Cynthia V. Rider and Kyle P. Messier, National Institute of Environmental Health Sciences (NIEHS)*

Humans and wildlife are routinely exposed to chemical mixtures from various sources, yet chemical risk assessments are traditionally completed chemical-by-chemical. Further, linking adverse outcomes to environmental chemical exposures is challenging, as real-world exposures involve multiple chemicals with unresolved spatial and temporal distributions. We have developed a geospatially resolved biological target-based risk assessment for exposure to chemical mixtures to address these challenges. This approach models the geospatial distribution of environmental chemicals based on the perturbation of a common molecular target. We exemplify this approach using the USA-wide National Air Toxics Assessment (NATA) and integrate these predicted external exposures at the county level. Using concentration-response data from curated high throughput screening (HTS) assays from Tox21 and ToxCast, we calculate a risk quotient by dividing each census tract's environmental chemical concentration by the activity concentration at cut-off (ACC). The individual risk surfaces are then combined into an aggregate risk surface by summing chemical risks that activate the same molecular target based on biological activity in a common HTS assay (e.g., CYP450 enzymes, estrogen receptor, etc.). The mapped results inform on the geospatial risk of molecular pathway perturbation from the exposure to the spatially resolved chemical mixture. Geospatial patterns that emerge will identify areas at a relatively elevated risk of molecular target perturbation based on combined environmental chemical exposures and chemical potencies. As a high throughput environmental screening tool, we have integrated these results into an R Shiny App, where the geospatial risk of molecular perturbation can be rapidly assessed for 643 high throughput screening assays and 83 individual NATA chemicals that occur in both datasets. This work will help advance risk assessments by providing a method for assessing the geospatial risk of exposure to environmental chemical mixtures and identifying regions with a higher cumulative risk of activating the selected molecular targets, which may lead to adverse health outcomes. Further, this geospatially informed risk assessment approach can be integrated with the adverse outcome pathway (AOP) framework by using the appropriate assays that can build a weight-of-evidence linking exposure to chemical mixtures with adverse outcomes, which may lead to adverse health outcomes.

## 3.05.P Wildlife Risk Assessment in the 21st Century: Integrating Emerging Science in Advancing Risk Assessment for Wildlife

### 3.05.P-We103 ToxChip Polymerase Chain Reaction (PCR) Arrays for Two Arctic-Breeding Seabirds: Applications for Regional Environmental Assessments

***Yasmeen Zahaby***[1]*, Pu Xia*[2]*, Doug Crump*[1]*, Jennifer F. Provencher*[1]*, Philippe Thomas*[1]*, Bruce Pauli*[1]*, Birgit Braune*[1]*, Ryan P. Franckowiak*[3]*, Michel Gendron*[1]*, Guy Savard*[1]*, Sailendra Nath Sarma*[1]*, Mark L. Mallory*[4] *and Jason M. O'Brien*[1]*, (1)Environment and Climate Change Canada, (2)University of Ottawa, Canada, (3) Queen's University, Canada, (6) Acadia University, Canada*

The Arctic is warming at a faster rate than anywhere else on earth due to climate change. This will lead to reductions in sea ice extent and permit increased shipping and oil extraction in areas such as the Baffin Bay/ Davis Strait (BBDS) region in Canada’s north. This poses a challenge for implementing evidence-based policies and wildlife monitoring initiatives to examine sublethal effects of oil-related contaminants on seabirds. We developed a toxicogenomics tool (ToxChip PCR array) to investigate background gene expression profiles for two Arctic-breeding seabirds; the thick-billed murre (Uria lomvia) and the black guillemot (Cepphus grylle). These novel tools will aid effects-based monitoring and can be rapidly employed after a spill event. ToxChip genes include those known to be sensitive to oil-related contaminants (i.e. polycyclic aromatic compounds [PACs]). Chemical burdens (53 PACs and 5 trace elements) and transcriptomic profiles (31 genes using a ToxChip PCR array) were examined in liver tissue (n = 30) of both species collected from the Qaqulluit and Akpait National Wildlife Areas in the BBDS region. The results provided benchmark gene expression data for two Arctic seabirds. Additionally, alkylated PACs and cadmium were the highest concentrated contaminants in both species. The biological pathways most impacted in both species were lipid homeostasis, thyroid hormone pathway and cellular metabolism. Future applications of the ToxChip include evaluating black guillemot gene expression in samples collected after a documented diesel-spill in Postville, NL and from a suspected natural seep in Qikiqtarjuaq, NU.

### 3.05.P-We104 Revision of the EFSA Guidance Document on Birds and Mammals: Industry view on positives and areas for clarification

***Thomas Bean***[1]*, Alex Blakey*[2]*, Markus Ebeling*[3]*, Manousos Foudoulakis*[4]*, Paula Garcia*[5]*, Sonja Haaf*[6]*, Joerg Hahne*[3]*, Steven Kragten*[7]*, Judith Neuwohner*[8]*, Joachim Nopper*[9]*, Dennis Sprenger*[9] *and Arnd Weyers*[3]*, (1) FMC Corporation, (2) Syngenta, United Kingdom, (3) Bayer CropScience LLC, Germany, (4) Corteva Agriscience, Greece, (5) Corteva Agriscience, United Kingdom, (6) ADAMA Deutschland GmbH, Germany, (7) Syngenta Ltd, Germany, (8) Nufarm Europe GmbH, Germany, (9) BASF SE, Germany*

The EFSA Guidance Document on the Risk Assessment for Birds and Mammals for the evaluation of Active Substances and Plant Protections Products, was completed at the end of 2009 and implemented for regulatory purposes in 2010 [EFSA, 2009)] (EFSA GD). After 12 years of extensive use by risk assessors in the EU, EFSA has launched the public consultation on the draft updated guidance document on Risk Assessment for Birds and Mammals [EFSA, 2021)].

The experience by industry (represented here by Crop Life Europe, CLE) on the scientific content and regulatory use of the EFSA 2009 GD has been presented in several well-attended conferences since 2009, including SETAC Europe. In this poster presentation, a major focus will be on advantages (e.g., new residue unit dose [RUD] values) and shortcomings (e.g., requirement to use $BMDL_{10}$ in place of NOEL) of the draft GD in the area of harmonization and to highlight topics that require further revision as the provided explanations are not conclusive and/or their implementation is not practical.

In the new draft EFSA 2021 GD update, it is very positive to see that a tiered approach is maintained from conservative to more realistic effects or exposure estimates. In addition, for Tier 1 we welcome the additional information provided for the selection of the toxicological endpoint, or the threshold for ecotoxicological relevance of effects.

On the other hand, the following needs to be further discussed before their implementation in routine risk assessment in the future: the new recommendations for the Benchmark Dose approach and the selection of the ecotox relevant reproductive endpoint, the use of the $f_{TWA}$ and agreed Tier 1 risk assessment. For the higher tier studies and modelling additional information was also provided but further clarity or discussions is required in some areas. Finally, it is appreciated that comments made on the EFSA GD back in 2017 were also considered.

Overall, the question to answer is whether the EFSA GD update serves its purpose to provide clarity at Tier 1 and higher tier, while also working towards the common goal of harmonised regulatory decisions that utilise our resources efficiently e.g., incorporate eco-modelling approaches, historical control data to improve protection of birds and mammals.

**3.05.V Wildlife Risk Assessment in the 21st Century: Integrating Emerging Science in Advancing Risk Assessment for Wildlife**

**3.05.V-01 Survey of new and emerging tools for wildlife ecological risk assessment.**
*Clare Lauren Kilgour[1], Elizabeth Power[1] and **Mark S. Johnson**[2], (1) Azimuth Consulting Group Inc., Canada, (2) U.S. Army Public Health Center*
In recent decades, the evolving science surrounding wildlife ecological risk assessment has produced a number of new and emerging tools with potential for application in risk assessment during the characterization of exposure, ecological effects and risk. Integrating these tools into risk assessment frameworks has the potential to improve risk estimates and potentially provide alternatives to animal-based methods. Understanding which of these new tools is ready for implementation and will provide data relevant to risk assessment is an important first step to integrate emerging science into existing risk assessment frameworks. The work presented in this poster follows-up on recommendations made in a series of papers being drafted for submission to SETAC journals. These recommendations span a range of tools that the authors believe would advance the practice of wildlife risk assessment in support of problem formulation, exposure assessment, effects assessment and risk characterization. To investigate these recommendations, and prioritize the recommended tools for application, we surveyed the authors and contributors of the papers. First, we reviewed the draft papers to compile a list of wildlife risk assessment tools believed to have promise to advance wildlife ERA. Second, survey respondents were asked to characterize each new tool for several metrics, corresponding to (1) the relevance of the tool with respect to use of the resulting data in risk assessment, and (2) the feasibility of applying the tool in a widespread and standardized manner. Survey results provide context for the readiness of each new tool for broad application, and support relative prioritization of the surveyed tools. Third, we analyzed the results of this prioritization survey to shed light on which of the emerging tools will provide the most value to wildlife risk assessment. The findings will be useful to support possible alternatives to animal-based methods and provide important context for future guidance, funding, and research on new and emerging tools in wildlife risk assessment. This poster will engage viewers to seek their input.

**3.05.V-02 Exploring publicly available economic and health-related data to identify biocides with potential to be emerging or priority environmental contaminants - two case studies relevant for aquatic wildlife**
***Sarah Marteinson**[1], Julie Anderson, Steven J. Cooke[2], Cory Dubetz[1], Michal Galus[1], Alexandria Green[1], Kerri Kosziwka[2], Michael Lawrence[1], Judith Leblanc[1], Ryan S. Prosser[3], Jessica L. Reid[2], Jessica Taylor[2], Zofia Taranu[4] and Amanda Winegardner[1], (1)Department of Fisheries and Oceans, Canada, (2) Carleton University,*

*Canada, (3) University of Guelph, Canada, (4) Environment and Climate Change Canada*
An important challenge faced by ecotoxicologists is determining what chemicals are currently and prominently used. Many new compounds are introduced into the market each year, and the use of established chemical ingredients changes in response to multiple factors including demand, innovation and regulation. Environmental concentrations from biotic or abiotic matrices are often relied upon to identify environmental contaminants where those with high or increasing concentrations are prioritized. However, the possibility of missing important contaminants exists if they are unknown or not widely monitored. Evidence is mounting regarding the potential for micropollutants to elicit biological effects and for rapidly metabolized contaminants to cause toxicity without accumulating in animal tissue, suggesting a reliance on environmental concentrations alone could cause important contaminants to be missed. In two recent case studies we evaluated how frequently-updated economic or health-based data could be used to identify and estimate the use of current biocides in Canada to inform prioritization of compounds for further analytical method development, aquatic biomonitoring and ultimately, wildlife effects research and risk assessment. In the first, pesticide use was estimated using the most recent annual sales data, which was available retroactively nationally and for some provinces. This information combined with environmental concentrations and known toxicity were used to prioritize pesticides with the most imminent need for further aquatic research. The second addressed similar goals but for a much less well-studied group of contaminants: antimicrobials. The same sources of information were unavailable and instead current-use disinfecting and sanitizing product lists (Health Canada) were consulted to identify active ingredients and use was estimated base on continuously available grocery store retail sales, import quantities (Statistics Canada) and the number of products containing the antimicrobials. These case studies provide examples of how multidisciplinary approaches and unconventional sources of information can be used in ecotoxicology, alongside traditional biomonitoring data. We found that different sources of economic and health-based data are available for different environmental contaminants; the pros and cons of each, recommendations for their validation and future directions will be discussed.

**3.05.V-03 GoMAMN: A Forum for Connection and Collaboration Focused on Values Driven Priorities for Bird Monitoring**

*Auriel MV Fournier[1], Evan Adams[2], Trey Barron[3], Janell Brush[4], Melody Chimahusky[5], Andrew Cox[4], Melanie Driscoll, Robert Dobbs[6], Jeffrey S. Gleason[7], Jacquelyn K. Grace[8], Clay Green[9], Houston Havens[10], Kevin Kalasz[7], Lianne Koczur[11], Joe Lancaster[12], Terri J. Maness[13],* ***Mary Ann Ottinger***[14]*, Jessica Schulz[15], Eric Soehren[16], Michelle Stantial[17], William Vermillion[7], Mark S. Woodrey[18], Kate Williams[2], Randy R. Wilson[7] and Theodore J. Zenzal Jr.[17], (1) Illinois Natural History Survey, (2) Biodiversity Research Institute, (3) Texas Parks and Wildlife Department, (4) Florida Fish and Wildlife Conservation Commission, (5) Mississippi Department of Environmental Quality, (6) Louisiana Department Wildlife & Fisheries, (7) U.S. Fish and Wildlife Service, (8) Texas A&M University, (9) Texas State University, (10) Mississippi Department of Wildlife, Fisheries, and Parks, (11) Alabama Audubon, (12) Gulf Coast Joint Venture, (13) Louisiana Tech University, (14) University of Houston, (15) New Hampshire Department of Environmental Services, (16) Alabama Department of Conservation and Natural Resources, (17) U.S. Geological Survey, (18) Mississippi State University*
Conservation planning for large, dynamic coastal and marine ecosystems has multiple benefits but is often challenging to implement. Prior to the Deepwater Horizon oil spill, avian monitoring projects frequently used study designs inconsistent with understanding species' population trends, species response to management actions (or restoration projects) and understanding ecological processes at larger scales. In addition, many research or monitoring efforts occurred at smaller spatial and temporal scales. Finally, there was little to no effort to coordinate across bird monitoring efforts such that target avian monitoring metrics could potentially be evaluated at larger spatial and temporal scales increasing relevance. In response to this, the Gulf of Mexico Avian Monitoring Network (GoMAMN) was created, to serve a forum for collaboration and coordination. To identify potential priorities for future bird monitoring efforts, GoMAMN used the principles of structured decision making to identify stakeholder objectives and values, which in turn led to the identification of specific

bird monitoring priorities. The Strategic Bird Monitoring Guidelines for the Northern Gulf of Mexico was an application of these goals into a series of monitoring guidelines designed to improve the utility of monitoring efforts. By using stakeholder objectives and values to identify bird monitoring priorities, practitioners and decision-makers have: (1) a set of agreed upon objectives and core values; (2) a transparent means of setting priorities across political and jurisdictional boundaries; and (3) a framework to facilitate communication and collaboration of data needs. Several of these priorities have already led to large collaborative co-production projects, which we will discuss in this presentation. We will also present on how GoMAMN is now working towards identifying what priorities we now need to focus time and energy, and better serving and responding to the broader Gulf of Mexico bird conservation community.

**3.06.P Poster Only: Terrestrial Toxicology, Ecology and Stress Response**

**3.06.P-Tu070 Metal accumulation and toxicity evaluation in terrestrial environment using wild rodents and lizards**

***Shouta M.M. Nakayama***[1]*, John Yabe*[2]*, Hokuto Nakata*[1]*, Rio Doya*[1]*, Kaampwe Muzandu*[2]*, Akifumi Eguchi*[3]*, Yared Yohannes*[1]*, Yoshinori Ikenaka*[1]*, Golden Zyambo*[2]*, Andrew Kataba*[1] *and Mayumi Ishizuka*[1]*, (1) Hokkaido University, Japan, (2) University of Zambia, (3) Chiba University, Japan*

Lead poisoning is often considered a traditional disease; however, the specific mechanism of toxicity remains unclear. Our research uses wild rat and lizard as sentinel animals to evaluate metal pollution levels in terrestrial environment. Study site in Kabwe, Zambia, is currently undergoing urban expansion, while lead contamination from former mining activities is still prevalent. In the present study, wild rodents collected from an area contaminated with lead (N = 18) and a control area (N = 10) were investigated. While the levels of plasma phenylalanine and isoleucine were significantly higher in a lead-contaminated area versus the control area, hydroxybutyric acid was marginally significantly higher in the contaminated area, suggesting the possibility of enhancement of lipid metabolism. In the interregional least-absolute shrinkage and selection operator (lasso) regression model analysis, phenylalanine and isoleucine were identified as possible biomarkers, which is in agreement with the random forest model. In addition, in the random forest model, glutaric acid, glutamine, and hydroxybutyric acid were selected. In agreement with previous studies, enrichment analysis showed alterations in the urea cycle and ATP-binding cassette transporter pathways. We also focused on a habitat generalist lizards (Trachylepis wahlbergii). The livers, lungs, blood, and stomach contents of 224 lizards were analyzed for their lead, zinc, cadmium, copper, nickel, and arsenic concentrations. Habitat types were categorized based on vegetation data obtained from satellite images. Multiple regression analysis revealed that land use categories of habitats and three other factors significantly affected lead concentrations in the lizards. Further investigation suggested that the lead concentrations in lizards living in bare fields were higher than expected based on the distance from the contaminant source, while those in lizards living in green fields were lower than expected. In addition, the lead concentration of lungs was higher than that of the liver in 19% of the lizards, implying direct exposure to lead via dust inhalation besides digestive exposure. Since vegetation reduces the production of dust from surface soil, it is plausible that dust from the mine is one of the contamination sources and that vegetation can reduce exposure to this. Further study will be implemented to understand molecular mechanisms of toxic effects in these terrestrial wildlife.

**3.06.P-Tu071 Understanding White-Nose Syndrome in Bats: Endohyphal Bacterial-Fungal Interactions of the Causative Agent of White-Nose Syndrome (Pseudogymnoascus destructans)**

*J. Ashton Reece, Alex S. Romer, **Kylie C. Moe** and Donald M. Walker, Middle Tennessee State University*

The pathogenic fungus *Pseudogymnoascus destructans* (Dikarya, Ascomycota, Thelebolales) causes white-nose syndrome of bats and has led to massive population declines in North American bat species. The outcome of bat infections by *Pseudogymnoascus destructans* depends on several facets of fungal physiology including the production of proteases and interactions with microbiome bacteria on the bat's skin. One of the most specific bacterial-fungal interactions occurs when endohyphal bacteria impact the potential niches their host fungi can colonize via manipulation of host fungal metabolism and enzymatic capacity. The objective of this project was to survey isolates of *P. destructans* for the presence of endobacteria, then characterize them by sequencing and quantifying phenotypic affects using clearing and comparative assays. We characterized a bacterial-fungal interaction between *P. destructans* and *Nocardia* sp. using molecular, physiological, and microscopic techniques. We found molecular and visual microscopic evidence of an endohyphal bacterium in the genus *Nocardia* of *P. destructans*. Fungal isolates were subjected to antibiotic treatment to reduce the population of endohyphal bacteria. Isolates that were released of their relationship with *Nocardia* had higher protease activity and were shown to have increased expression of the gene encoding a protease virulence factor SP1, which could imply *Nocardia* acts as an intracellular parasite to *P. destructans.* This work demonstrates the first endohyphal bacterial-fungal interaction in a wildlife pathogen and a likely antagonistic relationship between the bacterium and fungus.

**3.06.P-Tu075 The Acute Toxicity of Pesticide Mixtures to Honeybees**

***Jason B. Belden****, Oklahoma State University*

Honeybees *(Apis mellifera*) frequently live in complex environments where exposure to mixtures of pesticides is possible. Although several studies have expressed concern regarding the combined effects of pesticide mixtures, other studies did not find increased toxicity. Thus, the primary objective of this study was to identify peer-reviewed literature measuring the toxicity of pesticide mixtures to honeybees and determine how frequently synergistic interactions occur. Many experiments (258) were identified that met the criteria for inclusion. When considering all experiments, 34% of experiments had model deviation ratios (MDR; expected toxicity/observed toxicity) greater than 2, suggesting greater-than-additive toxicity. Twelve percent of experiments had MDR values greater than 5, with several studies exceeding 100. However, most experiments that had higher MDRs included azole fungicides or acaricides as a component of the mixture. After removal of these groups, only 8% of experiments exceeded an MDR of 2, and no experiments exceeded 5. Moreover, the influence of the azole fungicides was dose dependent. If only experiments that used azole exposure at environmentally relevant concentrations were considered, azole fungicides had limited impact on neonicotinoid insecticides. However, pyrethroid insecticides still had greater than expected toxicity with 80% of experiments having MDR values greater than 2. Acaricides also had greater than expected incidence of synergy with approximately 30% of studies reporting MDR values greater than 2. It should be noted that even the azole studies considered environmentally relevant frequently used maximum exposure rates and worst-case exposure scenarios. The primary finding is that synergy is uncommon except for a few cases where known synergists (azole fungicides) and pesticides with variable metabolism potential, such as some pyrethroids, are in combination. Future work is still needed to refine the relevance of azole fungicides at commonly occurring environmental concentrations.

**3.06.V Poster Only: Terrestrial Toxicology, Ecology and Stress Response**

**3.06.V-01 Terrestrial Risk Assessment of Pesticides in the Foumbot Municipality, West Region of Cameroon**

***Daniel Brice Nkontcheu Kenko***[1], *Norbert Tchamadeu Ngameni*[2] *and Nacaire Ahmed Njikam*[2]*, (1) University of Buea, Cameroon, (2) University of Dschang, Cameroon*

As crop yield is seriously hindered by pests, pesticide application in agriculture is inevitable with many implications on human and environmental health. This study aimed at assessing pesticide risk on bees, non-target arthropods (*Typhlodromus pyri* and *Aphidius rhopalosiphi*) and earthworms, using PRIMET (Pesticide Risks in the Tropics to Man, Environment and Trade), a pesticide risk model. For this purpose, data on pesticide application scheme in the study area and pesticide ecotoxicology was input into PRIMET one after the other to get the PEC (Predicted Exposure Concentration), NEC (No Effect Concentration) and ETR (Exposure Toxicity Ratio = PEC/NEC). The most dangerous pesticides for earthworms were acetamiprid (ETR=2963), glyphosate (ETR=1667) and imidacloprid (ETR=419). Compounds such as acetamiprid ($ETR_{bee}$=3252), cypermethrin

($ETR_{bee}$=487), emamectin benzoate ($ETR_{bee}$=278), imidacloprid (ETRbee=1383) and lambda-cyhalothrin ($ETR_{bee}$=295) are highly risky for bees. NTAs are particularly vulnerable to cypermethrin and imidacloprid, both of which exhibited the highest values of ETR of this study, 426 100 000 and 44 550 000, respectively. Other dangerous compounds for NTAs include chlorothalonil (ETR=2076), cymoxanil (ETR=1133), emamectin benzoate (ETR=1700), lambda-cyhalothrin (ETR=4900) and metalaxyl (ETR=2303). Imidacloprid, which posed risk to earthworms, bees and NTAs is a very risky compounds for the food chain. Acetamiprid was risky both for earthworms and bees, while cypermethrin, emamectin benzoate and lambda-cyhalothrin, posed risk both for bees and NTAs. Such very risky compounds should be avoided in agriculture and the use of less risky compounds should be implemented. Regulation on pesticide should be reinforced by decisionmakers and pesticides such as imidacloprid and cypermethrin, need particular attention.

**3.06.V-03 Exposure to Polycyclic Aromatic Hydrocarbons Induces Sex-Specific Transgenerational and Heritable Epigenetic Modifications in Drosophila melanogaster**

***Lindsey Annette Griffin***[1] *and Goran Bozinovic*[2]*, (1) Boz Life Science Research and Teaching Institute, (2) University of California, San Diego*

As environmental pollution is a prominent area of concern, it is critical to evaluate the potential heritability of the biological responses associated with adverse environmental exposures. Polycyclic aromatic hydrocarbons (PAHs) are considered one of the most widely distributed persistent organic pollutant (POP) in the environment, arising from incomplete combustion of organic substances, where they could cause health problems, such as various lung diseases while disrupting endocrine, reproductive, and digestive organ systems. The wide range of emission sources allows PAH pollution to be ubiquitous, as it accumulates throughout the atmosphere while integrating into terrestrial and aquatic environments. Our study aims to investigate the impact of PAH mixture exposure, identify sex-specific exposure responses, and provide mechanistic insights for explaining the effects and responses. Benzo[a]pyrene (B[a]P) is one of the most widely investigated PAHs, where exposures occur through inhalation, digestion, and direct dermal contact. Flies fed with regular food will be the negative control, exposure through B[a]P will be the positive control, and exposure through a standard PAH mixture will be the experimental condition. We will quantify sex-specific transcriptomic, epigenetic, and behavioral stress effects and responses and differentiate heritable vs. physiological effects using three generations of lab-reared and natural populations of fruit flies. Biological replicates separated as "families" will be grown in control media, where three replicates of the first generation are obtained. Once the fruit flies are sexually mature, female and male adult virgins will be combined and allowed to mate for approximately 48 hours before frozen at the 7-day adult stage, where the eggs of the next generation will develop. The natural population of fruit flies collected at Anza Borrego State Park, CA will be used to evaluate the translatability to natural populations frequently exposed to dynamic environmental changes. By modeling the exposure scenario of PAH pollution in the diet, we predict that upon transgenerational exposure, fruit flies will exhibit both common and sex-specific heritable epigenetic responses, evident through the correlation between behavior data, gene expression phenotypes, stress-induced DNA methylation and chromatin remodeling signatures.

**3.06.V-04 Poisoning bee: lethal effect of Imidacloprid on native brazilian bee Tetragonisca angustula**

***Aline Arantes de Oliveira***[1]*, Caroline Loureiro do Nascimento Silva*[1]*, Ana Carolina Veloso Oliveira*[1]*, Leticia Ferreira de Sousa*[1]*, Fernando Henrique Antoniolli Farache*[1]*, Marilene Silva Oliveira*[1]*, Aline Silvestre Pereira Dornelas*[2]*, Suzana Maria Loures de Oliveira Marcionilio*[1]*, Althiéris de Souza Saraiva*[1]*, Luiza Brum Rodrigues*[1] *and Isac Ricardo Rodrigues da Silva*[3]*, (1) Instituto Federal Goiano, Brazil, (2) Federal University of Tocantins, Brazil, (3)Instituto Federal de Educação Ciência e Tecnologia Goiano de Rio Verde, Rio Verde Goiás, Brazil*

Imidacloprid (IMI) is a neonicotinoid insecticide widely used to control insect pests in agriculture. This insecticide is persistence and toxic nature can affect foraging, olfactory learning and motor activity of bees, making it a potential pollutant for pollinators. Few studies evaluate the ecotoxicity of insecticides on native Brazilian bee species, which are important in the pollination of wild and cultivated plants. The present study aimed to assess the acute toxicity of IMI on the native Brazilian bee *Tetragonisca angustula*. Acute toxicity was evaluated through the Acute Topical Effect (ATE) and Acute Residual Effect (ARE) effect of the IMI-based insecticide on *T. angustula*. To determine the ATE effect, organisms were exposed to a known volume of mist (350 µL/min) nominal concentrations of 4, 8, 16, 32, 64, 128, 256, 512 µg a.i./L of IMI by 1 min. For ARE, organisms were exposed to contaminated food (sucrose-syrup) at the same concentrations as in ATE tests. The experimental design consisted in 10 organisms per cage with three replicates each, and the data were analyzed by Probit Analysis by Minitab 14 software. The LC10 and LC50 values were 8.97 and 85.84 µg a.i./L (respectively) for ATE and 10.05 and 50.30 µg a.i./L (respectively) for IMI ARE with 10.83 and 23.08 µg i.a./L (respectively) for ATE and 4.04 and 18.85 µg i.a./L (respectively) for Dimethoate ARE (analytical standard). Considering the importance of stingless bees as pollinating organisms in agroecosystems, our data draw attention to the effect of neonicotinoids, i.e., IMI, on survival of native bees subjected to agricultural pressure by insecticides.

We thank the Instituto Federal Goiano; Laboratório de Água e Efluentes and Conservação de Agroecossistemas e Ecotoxicologia, research group. Also, we are grateful to the Abelhando Mundo Afora project for the partnership and teachings - by Gabriel Benoski.

**3.06.V-05 The stingless bee Tetragonisca fiebrigi is more sensitive to the residual effect of Imidacloprid compared to the topical effect**

***Aline Arantes de Oliveira***[1]*, Althiéris de Souza Saraiva*[1]*, Daiany A Araújo*[1]*, Leticia Ferreira de Sousa*[1]*, Marilene Silva Oliveira*[1]*, Aline Silvestre Pereira Dornelas*[2]*, Suzana Maria Loures de Oliveira Marcionilio*[1]*, Fernanda dos Santos Farnese*[1]*, Bruno Dario*[1]*, Luiza Brum Rodrigues*[1]*, Luiz Ricardo Guimaraes Rezende de Oliveira*[1] *and Carlos Henrique Pereira Bento*[1]*, (1) Instituto Federal Goiano, Brazil, (2) Federal University of Tocantins, Brazil*

The native *Tetragonisca fiebrigi* are efficient pollinators in the neotropical region, and can be as environmental bioindicators, since they contribute to the balance and maintenance of the diversity of floristic communities in natural environments. However, these pollinating organisms have declined due to possible exposure to pesticides commonly used in the agroecosystem, such as neonicotinoids. Imidacloprid (IMI) has been reported to cause effects on non-targets pollinators, while the scientific community has highlighted the need for studies with native stingless bees. In this sense, we evaluated the acute toxicity, topical and residual effects of the insecticide based on IMI (Imidagold 700 WG®) on stingless bees *T. fiebrigi*. For this, the bees were placed in cages with a capacity of 250 ml for a period of 48 h, and submitted to eight nominal concentrations of insecticide in both trials (4, 8, 16, 32, 64, 128, 256, 512 µg a.i./L, plus control treatment - distilled water for topical effect and syrup (water and sugar) for the residual effect). They were fed 2 ml of the syrup. The experimental design consisted of 10 organisms per cage with three replicates each; data were analyzed by Probit Analysis using Minitab 14 software. In the topical assay, the toxicity of the LC10 and LC50 of IMI were observed at concentrations of 22.20 and 518.09 µg a.i/L, respectively. On the other hand, toxicity of residual effect were at 3.35 and 98.85 µg a.i/L IMI, respectively. Our study adds important information in the context of neonicotinoid ecotoxicity in stingless bees , since *T. fiebrigi* showed sign ificant sensitivity to IMI and the standard reference insecticide - Dimethoate, duly evidenced by LC10 (20.20 and 14.02 µg a.i./L) and LC50 (39.56 and 41.95 µg a.i./L), especially when ingesting contaminated food. Moreover, as there is a scarcity of scientific data on these pollinators, which are more frequent in the South, Midwest and Southeast regions of the Neotropics, this study begins to outline data on the effect of IMI on the survival of these invertebrates.

We thank the Instituto Federal Goiano; Laboratório de Água e Efluentes and Conservação de Agroecossistemas e Ecotoxicologia, research group. Also, we are grateful to the Abelhando Mundo Afora project for the partnership and teachings - by Gabriel Benoski.

**3.06.V-06 Effects of neonicotinoid-treated seed exposure on the earthworm Lumbricus terrestris**

***Elizabeth Brandt****, Sandra Schultz, Michelle L. Hladik, Michael Gross, Barnett A. Rattner and Natalie K.*

*Karouna-Renier, U.S. Geological Survey*
The most common application method of neonicotinoid insecticides in agriculture is through the treatment of seeds before planting, which is routinely utilized by farmers as an insurance measure even in the absence of pest pressure. Commercially formulated seed treatments are often sold as combination mixtures of various pesticides (e.g. insecticides and fungicides) along with other treatments (e.g., plant growth regulators), some of which are not well-represented in the published literature examining the effects of neonicotinoids on soil organisms. Despite the ecosystem services provided by soil organisms such as earthworms, the effect of pesticide-treated seeds on earthworms is not well understood. To address this data gap, we assessed the effects of treated wheat seed (coated with the neonicotinoid thiamethoxam and four fungicides) exposure on the deep-burrowing earthworm *Lumbricus terrestris* in a mesocosm. Using a multi-tiered approach, we examined changes in metabolomic profiles, acetylcholinesterase activity, oxidative stress indicators, and pesticide uptake and transfer to soil, plants and earthworms. Preliminary data analysis indicates that several metabolic pathways were altered in response to the treated wheat-seed exposure, while only minor differences were observed in oxidative stress indicators and acetylcholinesterase activity. Beyond the analysis of biological effects on the earthworm, data on pesticide residue and metabolite movement from the seed to soil, plants and earthworms are informative for assessment of potential neonicotinoid and fungicide hazard to earthworm predators and other wildlife.

## 3.07.P Late Breaking Science: Wildlife Toxicology, Ecology and Stress Response

### 3.07.P-Tu188 Temporal Variations in Perfluoroalkyl Substances (PFAS) in the Plasma of Smallmouth Bass and Associated Health Effects

***Heather L. Walsh** and Vicki Blazer, U.S. Geological Survey*
Centrarchids such as pumpkinseed, largemouth bass, bluegill, and smallmouth bass have been shown to accumulate high levels of PFAS and may have potential for use as indicator species of PFAS exposure.  In the Chesapeake Bay drainage, studies on smallmouth bass have identified testicular oocytes and vitellogenin in male fish as well as skin lesions, mortality, and population declines.  Previous work in the Chesapeake Bay drainage found associations with testicular oocytes and agricultural land use, animal feeding operations, and estrone.  More recently, a study identified PFAS in the plasma of smallmouth bass from four sites in two major tributaries (Potomac and Susquehanna River drainages) in the Chesapeake Bay.  Four PFAS (PFOS, PFUnA, PFDA, and PFDoA) were detected in every fish and land use at the two sites with the highest PFAS levels was dominated by agriculture.  Based on these findings, the objective of the current study was to expand upon this work by analyzing PFAS levels in the plasma of smallmouth bass from the same four sites with multiple types of land use (agriculture, urbanization, and forested) over multiple years and seasons.  Additionally, histopathology and liver transcript abundance analyses were conducted to identify pathological abnormalities (including parasite and macrophage aggregate abundance in multiple tissues) and changes at the molecular level that may increase our understanding of mechanisms associated with PFAS exposure.  With Nanostring nCounter® technology, liver transcripts associated with reproduction, oxidative stress, thyroid and immune function, and contaminant metabolism were quantified.  To date, this is the first temporal analysis of PFAS effects on biological endpoints in smallmouth bass from the Chesapeake Bay drainage.  The results will help management agencies identify priority regions where PFAS contamination needs to be addressed and/or further assessed for fish health and potential human consumption advisories.

### 3.07.P-Tu189 Toxicities of Legacy and Current Use PFAS in an Anuran: Do Larval Exposures Influence Responses to a Terrestrial Pathogen Challenge?

***Marisol S. Sepulveda**[1], Evelyn Barragan[1], Tyler D. Hoskins[1], Elizabeth Allmon[1], Jessica McQuigg[2], Matthew T. Hamilton[1], Erin Christian[1], Grace Coogan[1], Catherine Searle[1], Youn Jeong Choi[1], Linda S. Lee[1] and Jason T. Hoverman[1], (1) Purdue University, (2) Miami University*
Per- and polyfluoroalkyl substances (PFAS) are a large group of contaminants with strong carbon-fluorine bonds making them resistant to degradation. As such, legacy PFAS - perfluorooctane sulfonate (PFOS) and

perfluorooctanoic acid (PFOA) - are being replaced by alternative fluorinated compounds, such as hexafluoropropylene oxide dimer acid (GenX). These alternatives are thought to be less bioaccumulative and therefore, less toxic than legacy compounds that are known toxicants in anurans. In addition to anthropogenic pollutants, natural stressors including the fungal pathogen *Batrachocytrium dendrobatidis* (Bd) impact amphibian populations. Despite the co-occurrence of these stressors, no studies have examined interactive effects of Bd and PFAS. This study tested the growth and developmental effects of PFOS, PFOA, and GenX on gray treefrog (*Hyla versicolor*) tadpoles, followed by a Bd challenge in metamorphs. Results demonstrate that a PFAS larval exposure interacted with a terrestrial Bd challenge to influence growth and development. Bd exposed animals were significantly shorter with significantly increased body condition and mass. These are the first reported effects on amphibian terrestrial life stages after larval exposure to PFAS and increased sensitivity to Bd. The environmentally relevant concentrations tested here (0.1, 1. and 10 ppb) lend ecological significance to these results, however, additional mechanistic studies are needed.

**3.07.P-Tu190 Dietary Transfer of Mercury from Apple Snails to the Everglade Snail Kite in Central and Southern Florida, USA**

***Celeste Ortega-Rodriguez***[1]*, Brianne Soulen*[1]*, Aaron Roberts*[1]*, Ellen P. Robertson*[2]*, Robert J. Fletcher*[2] *and Anthony D. Sowers*[3]*, (1) University of North Texas, (2) University of Florida, (3) Bureau of Land Management*

Found in aquatic ecosystems, mercury (Hg) is a non-essential metal hazardous to wildlife. The Everglade snail kite (*Rostrahamus sociabilis)* is a federally endangered raptor known to exclusively feed upon freshwater apple snails (*Pomacea* sp.). However, concentrations of Hg in apple snails and snail kites has not been extensively studied. Here we determined concentrations of total Hg (THg) in the native Florida apple snail (*P. paludosa*), the invasive apple snail (*P. maculata*), and juvenile snail kite feathers across central and southern Florida to examine the relationship between Hg contamination in apple snails and juvenile snail kites. Samples were analyzed for Hg using a Milestone DMA-80 direct Hg analyzer. To estimate Hg exposure to Everglade snail kites, potential daily intakes of Hg ($DI_{pot}$) per body weight were calculated. In the present study, we found native and exotic apple snail THg concentrations were not significantly different. Mean THg for combined snail types was 94.80 + 49.76 ng/g ww (range: 12.25 - 278.91 ng/g ww). Mean THg concentration in Everglade snail kite feathers was 313.62 + 302.23 ng/g dw (range: 21.84 - 2380.9 ng/g dw). For sites with both apple snail and snail kite samples, we found a positive correlation ($r^2 = 0.8622$) between THg concentrations. Snails in the present study were divided into two shell size categories: 30 - 65 mm (preferred snail kite snail selection range) and > 65 mm (above snail kite snail selection range). Apple snails with shell lengths ranging from 30 - 65 mm had a mean THg concentration of 91.90 + 44.78 ng/g ww (range: 25.70 - 216.61 ng/g ww). Apple snails with shell lengths greater than 65 mm had a mean THg concentration of 97.04 + 53.61 ng/g ww (range: 12.25 - 278.91 ng/g ww). We found that THg concentrations for the two shell size categories did not significantly differ. For Everglade snail kites, $DI_{pot}$ were found to be below the LOAEL for snail kites foraging exclusively on apple snails ranging from 30-65 mm. However, $DI_{pot}$ may exceed LOAEL values if kites forage exclusively on snails over 65 mm. This study is the first to examine the relationship between Hg contamination in apple snails and snail kites. The results of the present study indicate that Hg can be dietarily transferred from apple snails to snail kites and suggest that snail kites foraging exclusively on large apple snails (>65 mm shell length) may be exposed to Hg concentrations that can pose a health risk to the Everglade snail kite.

## 3.07.V Late Breaking Science: Wildlife Toxicology, Ecology and Stress Response

**3.07.V-01 Translation of Terrestrial-Stage Amphibian Dermal Pesticide Exposures to Toxicity Endpoints Using a Dose-Response Framework**

***Elizabeth Paulukonis***[1]*, Jill Awkerman*[2]*, Robin Van Meter*[3]*, Matthew Etterson*[2]*, Donna Glinski*[4] *and Tom T. Purucker*[2]*, (1) Oak Ridge Institute for Science and Education (ORISE) participant at U.S. Environmental Protection Agency, (2) U.S. Environmental Protection Agency, (3) Washington College, (4) NRC Postdoctoral Fellow with U. S. EPA*

Dermal exposure of pesticides to terrestrial stage amphibians is largely understudied, particularly regarding dermal dose-response estimates that could inform population-level endpoints. At present, the ability to successfully develop terrestrial dermal dose-response curves depends on the structure and agreement of available data, and the utility of the datasets to capture applicable endpoints for pesticides of interest. Here, we synthesize available terrestrial stage anuran toxicity data and estimate dermal dose-response curves for a broad-spectrum herbicide and fungicide. We utilized ToxicR (an R-based version of the U.S. EPA's Benchmark Dose Software (BMDS 3.0)) in conjunction with a dermal exposure model based on pesticide application rates and terrestrial amphibian body weight to simulate curves under a Bayesian Markov Chain Monte Carlo (MCMC) approach, allowing us to quantify uncertainty in estimated curves and evaluate potential data gaps. We also compared outputs for LC50s derived from surrogate species to compare this approach with standard practices via the U.S. EPA's Web-ICE interspecies correlation method for risk assessment. Our analysis suggests that a) studies trend towards high (5-10x the label rate) exposure scenarios b) the approach produces viable toxicity curves for pyraclostrobin and glyphosate, and c) there is a significant gap in cohesive information regarding acute effects of dermal exposures for terrestrial stage amphibians. Our work details a first attempt at constructing a terrestrial stage amphibian dose-response curve for dermal exposures and describes the current state of available dermal exposure toxicity literature. We utilized the available datasets and methods to describe a straightforward workflow that can be leveraged alongside future attempts to assess ecological exposure and effects work.

**3.07.V-02 Multidisciplinary Approach to Determine the Influence of Global Changes on Nothotenia coriiceps**

*Lisette Zenteno-Devaud[1], **Gabriela V. Aguirre- Martinez**[2], María José Navarrete[3], Iván Hinojosa[1], Melissa Rebolledo[4], Fernada Vargas[1] and Isabel Aguirre[4], (1) Universidad Católica de Santísima Concepción, Chile, (2) Universidad Arturo Prat, Iquique, Chile, (3) Universidad de Ross, West Indies, (4) Universidad Austral, Chile*

Increasing human activities and ice-free areas expansion are the most prevalent threats facing Antarctic coastal fish. The effect of this threats depends largely on an understanding of the biology, ecology, distributions and feeding behavior of species. Therefore, a multidisciplinary approach is necessary to determine the influence of these disturbances. In this study, 30 *Nothotenia coriiceps* specimens were collected during the 2020 austral summer in two sites of Antarctic Peninsula (Fildes Bay and South Bay), contrasting in terms of anthropogenic pressure and natural processes. We used hematological parameters, body condition, isotopic signal ($\delta^{13}C$, $\delta^{15}N$) and the concentration of trace metals (Fe, Zn, Cu, Cd, Pb) to detect changes in health and ecology. While individuals captured in Bahía Sur exhibited higher concentrations of Cd ($P<0.005$, n=14), *N. coriiceps* from Fildes Bay showed high concentrations of Fe and Cu ($P<0.005$, n= 16). These results coincide with the concentrations recorded in the water column of each area, which, in turn, have been associated with: (1) Fe: loss of continental ice mass, (2) Cu: increases of contaminant sources (e.g. antifouling paint) (3) Cd: the presence of upwelling areas. In terms of individual alterations, specimens from Fildes Bay showed an increase in erythrocyte abnormalities and neutrophilia, likely reflecting xenobiotic damage and stress processes, respectively. Compared with *N. coriiceps* from South Bay, specimens from Fildes Bay exhibited significantly lower values in the Fulton index, high trophic diversity, and wide isotopic niches, suggesting sub-optimal diet conditions, diversification of feeding strategies, and consumption of prey with low nutritional value. Through a unifying mechanism, this study provides a foundation for understanding the alterations of Antarctic coastal fish as a result of global change. Funding: FONDECYT Nº3190455, N°11180466

## Track 4: Chemistry and Exposure Assessment

### 4.01 Addressing Exposure and Risk Associated with Chemical Contaminants in the Era of Big Data

**4.01.T-01 Addressing uncertainty in fundamental physical-chemical properties for environmental chemistry**

***Trevor N Brown***[1]*, Jon A. Arnot*[1,2,3] *and Alessandro Sangion*[1]*, (1) ARC Arnot Research & Consulting Inc., Canada, (2) University of Toronto, Canada, (3) University of Toronto Scarborough, Canada*

Physical-chemical properties are a fundamental aspect of environmental toxicology and chemistry, and chemical evaluations. These properties are key determinants of chemical fate in the environment, in organisms and in experimental test systems. Properties such as water solubility (WS), vapor pressure (VP), octanol-water partition ratio ($K_{OW}$), octanol-air partition ratio ($K_{OA}$), and air-water partition ratio ($K_{AW}$) are among the most commonly required data for understanding chemical fate and distribution in natural and artificial systems, and for determining the potential for adverse effects to humans and the environment. Compared to the vast number of chemicals produced and used by society, relatively few have complete high quality measurements of these fundamental properties. For many chemicals (e.g., those with low VP, low WS) technical methods are not adequate to measure these properties. Given the critically important nature of these chemical properties and significant data gaps there is a need to develop and validate reliable methods for their prediction. Uncertainty and applicability domain (AD) are vital considerations when considering measured or predicted properties to inform decision making. Quantitative Structure Property Relationships (QSPRs) and other estimation methods have been developed and applied for decades. Poly-parameter Linear Free Energy Relationships (PPLFERs) are also established tools for predicting physical-chemical properties of chemicals. PPLERs combine of system parameters, that describe the system properties, and solute descriptors, that depend on the chemical. Here we describe the development and validation of a new suite of predictive tools using the Iterative Fragment Selection (IFS) methods. The IFSQSARs provide explicit estimates of AD and uncertainty with predictions. We describe the general approach to developing and validating the IFSQSARs and compare their performance against other commonly used QSPRs (e.g., EPA's EPI Suite, OPERA models) and against empirical data. To facilitate applications and comparisons of the new IFSQSARs they have been implemented in the freely available on-line Exposure And Safety Estimation (EAS-E) Suite platform (www.eas-e-suite.com). These QSPRs have been integrated into the EAS-E Suite platform, which allows for the prediction of $K_{OW}$, $K_{OA}$, $K_{AW}$, VP and WS which can then be used to parameterize the various fate, toxicokinetic, bioaccumulation, exposure and risk models in EAS-E Suite.

**4.01.T-02 Curation Decisions and Statistical Methods for Large-Scale Ecological Risk Prioritization in Surface Water: Maximizing Incomplete and Non-Optimal Data**

***Risa Sayre***[1]*, Marc Serre*[1]*, Rhyne Woodrow Setzer*[2] *and John Wambaugh*[2]*, (1) University of North Carolina at Chapel Hill, (2) U.S. Environmental Protection Agency*

To prioritize chemicals for ecological risk assessment, it is necessary to understand at what concentrations chemicals are present in environmental media, such as in surface waters. Across the United States, hundreds of surface water chemical measurements are publicly available but this information often contains substantial data gaps. Additionally, in most records, only an upper limit is provided for a given sample (rather than a defined chemical concentration), the value of which may be many times greater than the hazard threshold for that chemical, particularly when the limit is not based on an analytical method but a regulatory reporting value. Given that the identification of potential risk considering environmental occurrence is more informative than determining risk solely based on hazard, and resources for environmental sampling are limited, there is a need to leverage available data to the extent possible. This project first explores data curation methods and inclusion decisions towards development of a final set for analysis, and second, statistical methods on the resulting selected records to estimate exposure values relevant for risk prioritization. Information level and content of each sample record, as well as the quantitative significance of different sample aggregations, were investigated. Using the results of these methods, we compare ranges of bioactivities across ecologically relevant species with ranges of exposure, resulting in categorical prioritizations for 350 chemicals The chemical deemed to pose the greatest ecological risk using this approach is relatively nontoxic but occurred in high environmental concentrations in many samples: a scenario which demonstrates the value of combining exposure and hazard data for such exercises. Overall, this work demonstrates the promise of incorporating otherwise under-utilized data in the risk assessment process and the effect of deciding which of those data to use and how. *This abstract does not necessarily reflect U.S. EPA perspective or policy.*

**4.01.T-03 Reconstruction of Chemical Exposures using Indoor Dust Levels: Evaluation with Measured Biomonitoring Data**

***Hyeong-Moo Shin***[1] *and Deborah H. Bennett*[2]*, (1) Baylor University, (2) University of California, Davis*

**Background/Aims:** Exposure to chemicals detected indoors is a growing public health concern because many of these chemicals have shown endocrine-disrupting or neurotoxic potential and people spend most of their time indoors. Settled indoor dust is a reservoir of many indoor chemicals. Our study objectives are (1) to reconstruct exposure to indoor chemicals by refining the currently available exposure assessment methods; and (2) to elucidate the extent to which indoor dust concentrations inform total body burden inferred from measured biomarker concentrations based on chemical properties and uses.

**Methods:** We selected chemicals whose measured concentrations are available in both biological samples ($C_{bio}$) of the U.S. National Health and Nutrition Examination Survey (NHANES) and an indoor exposome database previously developed through an extensive literature review (2,849 articles). First, we applied a dust-air partition coefficient ($K_{dg} = C_{dust}/C_g$) to estimate gas-phase concentrations ($C_g$), followed by a particle-air partition coefficient ($K_p = C_p/C_g$) to estimate particle-phase concentrations ($C_p$). Second, we forwardly modeled total intake rates (*iR*; µg/kg/day) by applying standard exposure factors to $C_g$, $C_p$, and $C_{dust}$. Third, we compared the modeled *iRs* with those inferred from NHANES biomonitoring data ('backward calculations'). Because this forward calculation method does not account for the contributions from food ingestion and application of personal care products, we also compared our results before and after integrating high-throughput (HT) dietary exposure predictions for chemical migrants from food contact substances and dermal exposure predictions for chemicals in personal care products.

**Results:** Overall, all modeled *iRs* from forward calculations were within two orders of magnitude of those from backward calculations; 72 of the 112 studied compounds were within one order of magnitude. The Spearman's correlation coefficient ($R_{sp}$) between the two *iR* calculation approaches was 0.73. For compounds with large octanol-air partition coefficients ($\log K_{oa}$ >10), a primary exposure route is modeled to be non-dietary dust ingestion.

**Conclusion:** Our study showed that residential chemical exposure can be reconstructed in a HT manner when biomarker measurements are lacking but indoor dust concentrations are available. Our results may be improved if concurrent measurements of environmental and biomarker concentrations are available from the same population.

**4.01.T-04 Should we assess the "mobility" of chemicals from a perspective of the "hazard" or "exposure"?**

***Zhizhen Zhang***[1]*, Alessandro Sangion*[2]*, Shenghong Wang*[1]*, Trevor N. Brown*[3]*, Jon A. Arnot*[4] *and Li Li*[1]*, (1) University of Nevada, Reno, (2) University of Toronto Scarborough, Canada, (3) TNB Research, Malaysia, (4) ARC Arnot Research & Consulting Inc., Canada*

Polar and ionizable organic chemicals are considered emerging threats to the environment and drinking water because of their high "mobility" in the environment: they can be transported over long distances, penetrate natural and artificial barriers, and resist removal by traditional water treatment procedures. Current chemical regulatory practices define chemical mobility based on the presence of chemicals in drinking water and the potential for human exposure. However, when screening the myriad of commercial chemicals for mobility, most existing efforts utilize bright-line criteria based on chemicals' intrinsic "hazard" properties, such as biodegradation half-lives for persistence (P) and organic-carbon-water partition coefficients for mobility (M). In this work, we compare and contrast the proposed intrinsic "hazard" criteria with chemicals' concentration in drinking water predicted by a fate and transport model, based on the data of 112,000+ discrete organic chemicals registered in different countries' chemical inventories. We evaluate the consistency of chemicals prioritized by "exposure" potential (chemicals with the highest concentrations in drinking water) and those by "hazard" assessment (chemicals with high P and M scores). We find that while chemicals with high P and M scores tend to possess a high potential to contaminate drinking water, P and M are not the only properties related to a chemical's potential to contaminate drinking water. An example is the low potential of volatile chemicals to contaminate drinking water, even if they have high scores in P and M. Thus, screening chemicals based on the P and M hazard indicators alone may, unfortunately, lead to "false positives" and "false negatives". It is therefore not appropriate to evaluate chemical mobility by using hazard indicators as a proxy. This work addresses the academic and regulatory need for a better understanding of the processes and properties related to drinking water contamination and for developing and evaluating scientifically defensible methods and criteria to identify and potentially regulate chemicals of concern for the safe and sustainable use of chemicals in commerce.

**4.01.T-05 An Integrated Approach to Identify the Toxic Contaminants Impacting an Endangered Beluga Whale Population**

***Holly Barrett***[1]*, Jonathan Verreault*[2]*, Magali Houde*[3]*, Frank Wania*[4] *and Hui Peng*[1]*, (1) University of Toronto, Canada, (2) University of Quebec at Montreal, Canada, (3 )Environment and Climate Change Canada, (4) University of Toronto Scarborough, Canada*

The beluga whales of the St. Lawrence Estuary (SLE) (Canada) were classified as endangered in 2014. Chronic exposure to contaminants is considered a major cause for the lack of population recovery and decline. While elevated tissue concentrations of pollutants have been reported in SLE belugas, the exact chemicals exerting toxicity remain unknown. Traditional targeted analysis and toxicity testing strategies have a limited capacity to identify the causative chemicals and may overlook unknown compounds. There is thus a pressing need to develop unbiased and robust methodologies to identify the contaminants posing risk to this population.

We applied nontargeted analysis to identify contaminants in SLE beluga tissue (liver and blubber) and reference Arctic beluga blubber. Peaks detected by electrospray and atmospheric pressure chemical ionization were matched to the EPA Tox21 database, and ~200 compounds were significantly more abundant in the SLE tissue, such as a bromoindole compound which we validated as an aryl hydrocarbon receptor (AhR) ligand.

Pooled liver and blubber extracts (100 $g_{tissue}$/mL) were tested in an *in vitro* AhR bioassay to investigate the contribution of known and unknown chemicals towards AhR-mediated responses. Significant responses were detected (*e.g.,* 0.16 g/mL of dosed liver induced AhR activity), marking the first time that AhR activity has been detected for beluga extracts. Notably, SLE blubber exerted significantly higher AhR activity than Arctic blubber, highlighting the unique chemical profile in SLE tissue which may be responsible for underlying nuclear receptor (NR)-mediated adverse health pathways. Effects-directed nontargeted analysis will be used to identify the chemicals responsible for the AhR activities.

Furthermore, the extracts were screened against a suite of 48 human NRs, revealing significant activity for several NRs including the estrogen, peroxisome proliferator-activated, and the Liver X receptors. Distinct from Arctic blubber, the SLE extracts exerted significant activity towards several orphan NRs with functions in cancer progression. Ongoing efforts are focused on characterizing the contribution of known and unknown chemicals towards NR activities using a NR-affinity pulldown approach. Ultimately, the results of this study will help fill critical knowledge gaps regarding the risks posed to the SLE beluga population and will support future conservation strategies.

**4.01.T-06 Plasma Protein Binding of 109 Per- and Polyfluoroalkyl Substances (PFAS): Using Category-Based New Approach Methods to Inform PFAS Toxicokinetics**

***Barbara A. Wetmore***[1], *Marci Smeltz*[1], *William Matthew Henderson*[1], *Matthew Scott Clifton*[1] *and Anna Kreutz*[2], *(1) U.S. Environmental Protection Agency, (2) Oak Ridge Institute for Science and Education (ORISE)*

New approach methods (NAMs) that utilize *in vitro* high-throughput screening data and *in silico* approaches to inform chemical safety assessment rely on *in vitro* toxicokinetic (TK) data to enable translation of bioactive *in vitro* assay concentrations to exposure metrics reflective of administered equivalent doses. With 1,220 per- and polyfluoroalkyl substances (PFAS) listed on the U.S. Toxic Substances Control Act (TSCA) inventory and growing concern over their widespread presence and persistence in the environment, the utility of NAMs to predict the potential exposures and toxicokinetics across these data-poor yet structurally diverse compounds is now being evaluated. To address the deficiency in TK data, 143 PFAS stocks, selected to span a wide range of functional groups and physico-chemical properties and after passing a quality control assessment, were evaluated for *in vitro* human plasma protein binding (PPB) using ultra high-performance liquid chromatography (UPLC) or gas chromatography (GC) coupled with tandem mass spectrometry (MS/MS). PPB was measured in 10-donor mixed sex pools of human adult plasma using ultracentrifugation, a membrane-less approach that separates plasma aqueous fraction from other plasma constituents by centrifugation at 850,000xg for 4 hours. Fraction unbound ($f_u$) values were successfully measured for 109 PFAS: 65 by UPLC-MS/MS and 44 GC-MS/MS. Median, 25$^{th}$ and 75$^{th}$ percentile $f_u$ values for the 109 were 0.0230, 0.0039, and 0.1003, respectively, with the lowest $f_u$ (0.0001) recorded for pentadecafluorooctanoyl chloride ($C_8ClF_{15}O$). Trend analyses across a range of properties indicated associations between increasing chain length and lower $f_u$ (i.e., higher binding) and low van der Waals volumes with higher $f_u$. Evaluations using ToxPrint ChemoTypes for grouping indicate lower binding for PFAS comprised of alcohol, aliphatic amine, and amino-carbonyl groups compared against PFAS carboxylic acids and sulfonic acids. Binding comparisons across other groupings and the entire tested PFAS space are also presented. With high PPB associated with bioaccumulation across all species, these findings demonstrate potential utility of a NAM read-across approach to estimate PFAS human and ecological risk for the broader data-poor PFAS domain. *The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the US EPA.*

**4.01.T-07 Precision Environmental Health Monitoring by Longitudinal Exposome and Multi-Omics Profiling**

***Peng Gao***[1], *Xiaotao Shen*[1], *Xinyue Zhang*[1], *Chao Jiang*[2] *and Michael Snyder*[1], *(1) Stanford University, (2) Zhejiang University, China*

Conventional environmental health studies primarily focused on limited environmental stressors at the population level, which lacks the power to dissect the complexity and heterogeneity of individualized environmental exposures. Here we integrated deep-profiled longitudinal personal exposome and internal multi-omics to systematically investigate how the exposome shapes a single individual's phenome as a pilot case study. We annotated thousands of chemical and biological components in the personal exposome cloud and found they were significantly correlated with thousands of internal biomolecules, which was further cross validated using corresponding clinical data. Our results showed that agrochemicals and fungi predominated in the highly diverse and dynamic personal exposome, and the biomolecules and pathways related to the individual's immune system, kidney, and liver were highly associated with the personal external exposome. Overall, this data-driven longitudinal monitoring study demonstrates the potential dynamic interactions between the personal exposome and internal multi-omics, and the impact of the exposome on precision health by producing abundant testable hypotheses.

**4.01.T-08 Poster Highlights: Addressing Exposure and Risk Associated with Chemical Contaminants in the Era of Big Data**

***Li Li***, *University of Nevada, Reno*

We live in a chemical-intensive world where the number of substances on the market is constantly growing.

Exposure to a multitude of these chemicals can be associated with adverse impacts on human health and ecosystems. Clearly, conventional, hypothesis-driven, single-chemical assessment practices are inadequate to address the exposure and risk associated with such large amounts of environmental chemicals. Moreover, most of these chemicals are data-poor, which requires models and data techniques to fill data gaps. In this poster highlights time slot, the authors of some of the excellent posters submitted to the session will provide an overview slide presentation to highlight the major findings in their poster presentation. These posters showcase methodologies, techniques, and applications of big data in chemical assessment and management.

**4.01.P Addressing Exposure and Risk Associated with Chemical Contaminants in the Era of Big Data**

**4.01.P-Th072 Machine Learning to Integrate Environmental Modeling and Monitoring Data**
*Yaoxing Wu, **David A. Dreier**, Lula Ghebremichael, Nicholas Geitner and Wenlin Chen, Syngenta*
Environmental modeling and monitoring both play a critical role in pesticide regulatory assessments. Standard scenario-based mechanistic models such as the U.S. Environmental Protection Agency (EPA) Pesticide Water Calculators (PWC) are almost exclusively used for ecological and drinking water exposure assessments. Surface water monitoring data when satisfying certain data quality criteria can be used in the new EPA pesticide drinking water assessment paradigm through a time-series regression (SEAWAVE-QEX). These two approaches (i.e., scenario-based PWC and monitoring-based SEAWAVE-QEX) are implemented in tiers, but little attempt has been made to connect them, resulting in limited opportunity for pesticides without or with less abundant monitoring data to be considered for high tier refinements. In this paper, we propose and show how the data-driven Machine Learning (ML) model can aid connecting a mechanistic model PWC and/or SWAT (Soil Water Assessment Tool) with water monitoring data and bring in real watershed scale drivers into the refinement of water exposure predictions. We use the most intensively sampled atrazine water monitoring data to train and test the ML model. Measured concentrations serve as target variable in ML modeling. Predictions of scenario-based models (PWC or SWAT) are incorporated into the ML datasets as proxy feature variables which reflect the insight of the mechanistic processes of chemical fate and transport and other variables including physico-chemical properties and label-prescribed use patterns. Scenario-based models may or may not accurately represent the specific watershed scale fate and transport processes. This gap between standard scenarios and watershed-specific features are bridged through ML modeling. Model performance is evaluated by the measured atrazine concentrations and a series of cross evaluation and statistical criteria. Examples of using such an approach are provided.

**4.01.P-Th074 Empirical measurement of PFAS dosing within in vivo aquatic high throughput assays**
***Brett R. Blackwell**, Kendra Bush, Kevin Flynn, John Hoang, Michelle Le, Emma Stacy and Daniel L. Villeneuve, U.S. Environmental Protection Agency*
As part of the US EPA's *PFAS Strategic Roadmap*, the agency is committed to increasing our understanding of the potential ecological effects of per- and polyfluoroalkyl substances (PFAS). To improve the pace of PFAS chemical assessments, new approach methods focused on ecologically relevant aquatic species have been developed, allowing a more rapid assessment of chemical toxicity by high throughput testing (HTT). Moving to HTT assays does create additional challenges for exposure characterization, and as a consequence, most HTT data are reported only for nominal exposure concentrations; however, unique chemical properties can lead to substantial deviations between reported nominal concentration and the free concentration in solution. While in vitro chemical distribution models exist, most are applicable only for neutral organics, whereas PFAS – most of which are ionogenic chemicals – fall outside of model domains of applicability due to their unique physicochemical properties. As such, empirical measurements of PFAS in HTT systems are currently needed to accurately determine exposure conditions within in vivo HTT assays. Here, we empirically measured the concentration of 22 PFAS in exposure media following 24 h exposure of individual PFAS to either *Pimephales promelas* larvae or juvenile *Daphnia magna* in a 96 deep well format. After exposure, aliquots of exposure media were removed and stabilized with an equal volume of acetonitrile. All analytes were subsequently

measured by HPLC-MS/MS. Tested PFAS included multiple homologues of carboxylic and sulfonic acids, sulfonamides, and fluorotelomer sulfonates, among others. In general, homologues followed a predictable pattern of decreasing free concentration with increasing chain length (e.g., ranging approximately 70 – 1.0% of nominal for 5 – 14 chain carboxylic acids). A more volatile fluorotelomer alcohol showed less than 1% recovery likely indicating loss to headspace and/or method incompatibility, while a fluoroether compound was fully degraded prior to exposure likely due to initial stock solubilization in DMSO. The results highlight the need to fully consider the physicochemical properties of specific PFAS prior to HTT assay screening. Data will be presented and discussed as it pertains to interpreting HTT effects data and to the development of models for PFAS distribution within in vivo HTT assays. *The contents of this abstract neither constitute nor necessarily reflect US EPA policy.*

**4.01.P-Th075 Evaluating several in vitro disposition models for use in high-throughput toxicokinetic research**

***Steven Lasee***[1]*, Johanna Nyffeler*[2]*, Brett R. Blackwell*[2]*, Joshua Harrill*[2]*, Felix Harris*[3]*, Jonathan Haselman*[2]*, John Nichols*[2] *and Daniel L. Villeneuve*[2]*, (1)Oak Ridge Institute for Science and Education (ORISE) participant at U.S. Environmental Protection Agency, (2) U.S. Environmental Protection Agency, (3) ORAU student contractor at U.S. Environmental Protection Agency*

In vitro high-throughput screening assays are being increasingly adopted as part of a tiered testing strategy for chemical hazard evaluation. As such, it has become important to understand chemical behavior within in vitro assay systems to accurately predict the bioavailable chemical concentration at a calculated nominal in vitro potency. Several in vitro distribution models have been developed to predict chemical partitioning using physiochemical properties along with assay specific parameters including well dimensions, cell density, and serum protein content. The purpose of this study was to use available models to predict chemical distribution in a 384-well format version of OECD test guideline 249 (OECD TG249) for cell viability testing in rainbow trout gill cells (RTgill-W1). A total of 231 environmentally relevant chemicals were chosen for screening in the assay based on available in vivo rainbow trout toxicity, in vitro toxicity, or known environmental occurrence. Final model outputs will be applied to the nominal point-of-departure (POD) to calculate a free chemical concentration, which will then be used for in vitro-to-in vivo extrapolation. Currently, assay specific model input parameters have been determined, and in vitro distribution estimates were generated for 201 of our 231 test chemicals using the Armitage 2014 model. The remaining 30 chemicals, which included polymers, ionic compounds, and metals, were excluded due to model incompatibility. The log $K_{ow}$ for input chemicals ranged -2.63 to 7.61, and the fraction of freely dissolved chemical in cell media ($F_{free}$) ranged 0.0002 to 1.0. The Armitage 2014 model is largely $K_{ow}$ dependent and predicted $F_{free}$ in this assay drops below 0.5 at a log $K_{ow}$ of approximately 3.9. Future outputs from the Fischer 2017, Fisher 2019, Armitage 2014, and Armitage 2021 models will be compared to identify chemical domains showing agreement (or lack thereof) across the different modeling approaches. Ultimately, the modeled chemical distribution will be compared with empirically measured concentrations of a subset of 12 chemicals to assess model performance and determine which model(s) best describe chemical behavior within this in vitro system to more accurately calculate PODs using in vitro-to-in vivo extrapolation. *The contents of this abstract neither constitute, nor necessarily reflect, official US EPA policy.*

**4.01.P-Th076 Understanding Inter-Individual Variability in Short-Chain Chlorinated Paraffin Concentrations in Human Blood**

***Shan Niu***[1]*, Xi Chen*[2]*, Ruiwen Chen*[1]*, Yun Zou*[3]*, Zhizhen Zhang*[4]*, Li Li*[4]*, Kimberly J. Hageman*[5]*, Carla A. Ng*[1] *and Liang Dong*[6]*, (1) University of Pittsburgh, (2) Chinese Center for Disease Control and Prevention, (3) University of Liege, Belgium, (4) University of Nevada, Reno, (5) Utah State University, (6) National Research Center for Environmental Analysis and Measurement*

Chlorinated paraffins (CPs), particularly short-chain CPs (SCCPs), have been reported in human blood samples with high detection frequency and high variation among individuals. However, factors associated with and their contributions to inter-individual variability in SCCP concentrations in human blood have not been comprehensively assessed. This study investigated the possible key factors driving the variation of SCCP concentrations in human blood. We measured SCCP concentrations in 57 human blood samples collected from a cohort of individuals living in two adjacent villages in Fuyang City, China using comprehensive two-dimensional gas chromatography coupled with electron capture negative ionization mass spectrometry. We then used the PROduction-To-Exposure (PROTEX) model to investigate the extent to which sociodemographic data, biotransformation rates, dietary patterns, and indoor contamination could explain the observed inter-individual variability in blood SCCP concentrations. SCCPs were detected in all human blood samples with concentrations ranging from 122 to 1233 ng/g, wet weight. Measured ∑SCCP concentrations varied by a factor of nine in males and a factor of 10 in females. Modeled ∑SCCP concentrations ranged over a factor of two in both males and females, correlated to variations in age, sex, and body weight. Similarly, modeled ΣSCCP concentrations varied by a factor of 3 in males and 2 in females correlated with variation in dietary composition. Variations in the modeled ΣSCCP concentrations increased to factors of 6 for males and 5 for females by assuming inter-individual variability in biotransformation rates of a factor of 3. In addition, modeled ΣSCCP concentrations varied by a factor of 8 for both male and female groups when variability in indoor contamination was considered. Based on comparisons of measured data and modeled scenarios, inter-individual variabilities in human biotransformation and indoor contamination may be the two key factors driving the variation of SCCP concentrations in human blood. This study helps in identifying subpopulations that are most susceptible and vulnerable to environmental pollution, to be selected for exposure intervention. In addition, such information also helps computational exposure scientists and toxicologists understand how uncertainty is propagated in toxicokinetic and risk models. Future work is needed to confirm this conclusion.

**4.01.P-Th077 Predicting the chemical properties of mixtures and mixture components from chemical structure with QSPRs and PPLFERs**

***Trevor N Brown***[1]*, Jon A. Arnot*[1,2,3]*, Alessandro Sangion*[1]*, Alena Celsie*[4] *and J. Mark Parnis*[4]*, (1) ARC Arnot Research & Consulting Inc., Canada, (2) University of Toronto, Canada, (3) University of Toronto Scarborough, Canada, (4) Trent University, Canada*

Developing a better understanding and improved predictive tools for mixtures has been identified as a key research need in the field of chemical risk assessment. Poly-parameter Linear Free Energy Relationships (PPLFERs) are a well-established tool in environmental chemistry for predicting partitioning and transport properties of chemicals. PPLFERs are composed of system parameters which characterize the solvation environment in which partitioning takes place, and solute descriptors which characterize the chemical of interest. The first major limitation of applying PPLFERs to chemical risk assessment is that hundreds of thousands of chemicals need to be assessed, but there are only solute descriptors for a few thousand chemicals, and system parameters for about one hundred solvent-air and solvent-water partitioning systems. In most mixtures, the chemical components act as both solutes and solvents, so solute descriptors and system parameters need to be known for all mixture components. In this research, empirical correlations were developed to predict system parameters from experimental solute descriptors, and reliable solvent-air partitioning system parameters were predicted for about one thousand chemicals. Quantitative Structure Property Relationships (QSPRs) were then created for system parameters using this expanded dataset, along with QSPRs for solute descriptors. These developed PPLFER and QSPR tools can predict the behaviour of liquid chemicals in a mixture as both a solute and solvent, and gaseous and solid minor components can be included as solutes. A new model for predicting the Vapor Liquid Equilibrium (VLE) of mixtures has been developed based on a modification of the log-linear co-solvency model of Yalkowsky with the solute descriptors and system parameters of the mixture components as inputs. A new model for predicting the skin permeability ($K_P$) of mixtures applied to the skin has also been developed based on previous PPLFERs for $K_P$ of pure chemicals developed by Abraham, with solute descriptors, system parameters and VLE of the mixture components as inputs. These tools have been made available as a part of the freely available on-line Exposure And Safety Estimation (EAS-E) Suite platform (www.eas-e-suite.com).

**4.01.P-Th078 Addressing uncertainty in toxicokinetic data and applications to advance chemical exposure and risk assessment**

***Alessandro Sangion***[1,2]*, James M. Armitage*[3] *and Jon A. Arnot*[2,4]*, (1) University of Toronto Scarborough, Canada, (2) ARC Arnot Research & Consulting Inc., Canada, (3) AES Armitage Environmental Sciences, Inc, Canada, (4) University of Toronto, Canada*

Quantitative knowledge of toxicokinetic (TK) processes is necessary to better understand relationships between external and internal exposures of chemicals. New Approach Methodologies (NAMs) such as in vitro-in vivo extrapolation (IVIVE) and high-throughput toxicokinetic (HTTK) modeling can be used to obtain TK data necessary for chemical priority setting, screening, and risk assessment. However, there is the need to systematically examine existing data and NAMs to foster confidence in their application. The objective of this work is to use high quality data and models to perform quantitative comparisons of TK information between different in vitro test systems (i.e., S9, Microsome and Hepatocytes), different levels of organization (i.e., in vitro and in vivo), different species (e.g., fish, rodent, human), and between routes of exposure (e.g., oral and inhalation). These comparisons are based on curated in vitro and in vivo TK databases and IVIVE and HTTK models included in the Exposure And Safety Estimation (EAS-E) Suite platform. The EAS-E Suite databases include in vitro (> 9000 chemicals) and in vivo (> 1100 chemicals) TK data in different species (i.e., fish rodent, human) for thousands of chemicals (e.g., hydrocarbons, chemicals in personal care products and consumer items, halogenated flame retardants etc.). Recently validated Quantitative Structure Activity Relationship (QSAR) models to predict in vitro intrinsic clearance and in vivo biotransformation half-lives were included in the analysis. Finally, the reverse TK (rTK) workflow embedded in EAS-E suite has been used to extrapolate in vitro data to in vivo data and compare multiple data streams. Various exploratory analyses have been performed including the critical evaluation of in vitro and in vivo TK data, the comparison of in vitro data by assay and species, the interspecies analysis of in vivo data to explore potential allometric (body mass) relationships and potential interspecies differences, and the comparison IVIVE data with in vivo empirical data within and across species. The results of these analyses showed encouraging correlations and trends in the TK data from different species highlighting how critical evaluation of the existing data and models can help address uncertainty in TK data.

**4.01.P-Th079 Predicting Chemical Tendencies Within the Human Body**

***Amy Olsen****, Li Li and Dingsheng Li, University of Nevada, Reno*

A systematic understanding of the dosimetric relationship between the human intake of a chemical contaminant and its concentration in bodily fluids is critical for assessing human exposure and health impacts associated with chemical production and use. However, it remains less clear how such a dosimetric relationship depends on the interaction between fundamental chemical properties and human physiological features. In this work, the dosimetric relationship is characterized by a ratio of the daily chemical intake dose to the steady-state concentration in the human blood, which quantifies the chemical's tendency of accumulation within the human body. Using a well-evaluated model, this work investigates the dependence of such a dose-to-concentration ratio on properties of partitioning between octanol, air, and water (i.e., the hydrophobicity and volatility of chemicals) and age-dependent body composition, by linking it to toxicokinetic processes that determine the absorption and elimination of chemicals. These investigations are visualized in a series of chemical partitioning space plots, which inform us of the chemicals that, once in the body, are likely to be absorbed by the body and how long they are predicted to persist within the body. Our results indicate that chemicals with moderate hydrophobicity and relatively low volatility are most accumulative in the human body, owing to their efficient absorption and inefficient elimination. Another finding is the applicability of the ratio outcome to both adults and children with little variance between the two. Overall, this study is important in helping to provide a way to predict blood concentrations of a chemical if the information is available for exposure (e.g., estimates from the U.S. EPA's ExpoCast project), and to back-calculated the rate of exposure that sustains the blood concentrations observed in biomonitoring campaigns (e.g., measurements from NHANES). The results could be used to help those who set or advocate for safe environmental regulations as well as pharmacists and others working within this field. The model can also be used as a useful New Approach Method (NAM) tool by environmental toxicologists, exposure scientists, biomonitoring practitioners, and health risk assessors.

**4.01.P-Th080 A Refined Physiologically Based Pharmacokinetic Model for Perfluorooctanoic Acid (PFOA) In Zebrafish**

***Yuexin Cao**, Shan Niu, Manoochehr Khazaee, Patrick Treacy and Carla A. Ng, University of Pittsburgh*

Per- and polyfluoroalkyl substances (PFAS) are a class of synthetic chemicals with useful surface-active properties and resistance to harsh environments. They have wide industrial and commercial applications, such as in semiconductors, firefighting foams, non-stick cookware and paper/paperboard packaging. The extensive applications provide more opportunities for PFAS entering the environment and organisms. After entering organisms, PFAS have the potential to accumulate in various tissues, including blood, liver, kidney and brain, leading to potential toxic effects. Zebrafish (*Danio rerio*) is a widely used vertebrate model for exploring the toxicological characteristics of chemicals. To enhance the potential of zebrafish as a "model" organism, more studies including toxicokinetic modeling is needed. Physiologically based pharmacokinetic (PBPK) models are powerful tools to simulate the absorption, distribution, metabolism and excretion (ADME) of xenobiotic substances. However, there are limited PBPK models for zebrafish, especially in terms of PFAS. In this study, we updated a previous zebrafish PBPK model of perfluorooctanoic acid (PFOA) in zebrafish developed by our group in 2018. PFOA was selected as a representative PFAS due to greater data availability. We first performed a literature search from 2018 to 2022 to update zebrafish-specific physiological and PFOA-related parameters needed in the PBPK model. For those parameters that lacked zebrafish-specific data, we estimated and adjusted parameters based on studies of other fish or, at the worst case, mammals. Then the model performance was evaluated by comparing experimental data with modeled ones. We used a method developed alongside the 2018 model to score parameter quality based on method of collection and the species they were derived from. Finally, Monte Carlo analysis was conducted to evaluate the uncertainty and sensitivity of the model and identify critical data gaps. Our study helps to better predict the distribution of PFOA in various tissues of zebrafish by updating the model with the latest available parameters and identifies critical parameters that affect the performance of this PBPK model.

**4.01.P-Th081 Review and comparison of QSAR/QSPR models in chemical mobility assessment**

***Alessandro Sangion**[1,2], Zhizhen Zhang[3], Trevor N Brown[2], Jon A. Arnot[2,4] and Li Li[3], (1) University of Toronto Scarborough, Canada, (2) ARC Arnot Research & Consulting Inc., Canada, (3) University of Nevada, Reno, (4) University of Toronto, Canada*

Persistent, mobile, and toxic (PMT), and very persistent and very mobile (vPvM) chemicals are perceived as an emerging threat to aquatic environments and drinking water quality. The combination of persistence and mobility increases the chance for substances to be efficiently transported from the sources of release to groundwater or remote aquatic ecosystems, through passing natural barriers and water treatment facilities. Environmental fate and exposure models can be used to identify persistent and mobile chemicals by quantifying their environmental fate and exposures and characterizing their ecological and human health risks. Validated quantitative structure activity/property relationship (QSA(P)R) models can be used to support the efficient and defensible parameterization of the chemical fate and exposure models. This work reviews QSA(P)Rs for persistence and sorption to sediments, soils, and suspended solids that are critical for addressing uncertainties associated with persistence and mobility assessment.

Few QSA(P)R models have been developed to predict the persistence of chemicals in the aquatic environment, e.g., through biodegradation and hydrolysis. QSARs for predicting environmental biodegradation half-lives are essentially not yet available for chemicals other than petroleum hydrocarbons. Only qualitative classification models for ready biodegradability are available in user friendly software such as BIOWIN, OPERA and VEGA. QSA(P)Rs for hydrolysis are generally based on the variation of the Hammet and Taft equations using a combination of polar and steric factors and focus on the reactivity of specific groups of compounds. Such

QSA(P)Rs have significant limitations and can only be applied to a limited type of chemicals under certain conditions. Furthermore, QSPRs for sorption to sediments, soils, and suspended solids are generally developed for $K_{OC}$ (adsorption coefficient normalized to the organic carbon (OC) content) based on empirical relationships with octanol-water partition ratio ($K_{OW}$) or distribution ratio ($D_{OW}$). However, $K_{OW}$ and $D_{ow}$ alone are not mechanistically sound predictors for the sorption process, especially for ionogenic organic compounds (IOCs) where specific interactions between IOCs and sorbents are not determined by hydrophobicity alone. Overall, there is a lack of comprehensive or generic QSA(P)R models for predicting endpoints related to persistent and mobile chemicals, especially for hard-to-test substances such as IOCs.

**4.01.P-Th082 Air quality and social perception in a University Campus. Guadalajara, Mexico**

*Javier Omar Martínez Abarca, **Martha Georgina Orozco Medina**, Arturo Figueroa Montaño and Valentina Davydova Belitskaya, Universidad de Guadalajara, Mexico*

In urban spaces there is a variety of factors that exert constant pressure on the landscape and the environment and affects at local, regional and international levels. Exposure to air pollution is linked to work occupation that occurs through direct contact with emission sources, which are a health risk, particularly to the respiratory system. Air quality was studied with the installation of a monitor outside the buildings that are located near the busiest avenues that adjoin the Campus. The equipment records minute measurements from which hourly averages were determined. The measurement period was from January 4 to March 28, 2021 and the pollutants that were monitored were $CO_2$, VOC, particulate matter ($PM_{2.5}$ and $PM_{10}$ µm), in addition to meteorological variables of temperature and humidity. The results were compared with Mexican air quality guidelines and standards of the World Health Organization (WHO), as well as those reported in the scientific literature. The global hourly analysis showed a different pattern between the concentrations of $PM_{2.5}$ and $PM_{10}$ compared with data from local network stations of the State's Monitoring System. Unusual increases were registered during the night and decreases during the middle of the day. This pattern may possibly due to the working activities of the New Macrobus Transportation System, wich is taking place in the peripheral ring of the Metropoly. The perception of the population was studied with the application of a modified survey covering the issues: sociodemographic, perception of air pollution and health risk, noise and its relationship to COVID-19. The results showed the importance of noise, odour and dust pollution with healthy atmosphere in the work place.

**4.01.P-Th084 Early Life Exposure of Zebrafish to the Neonicotinoid Insecticide, Imidacloprid**

***Janine Fetke**[1], Jordan Crago[2], Weichun Huang[1], Mary Jean See[1], Morgan Hu[1], John Martinson[1], Audrey Willis[2], Seenivasan Subbiah[2], David Bencic[1], Robert Flick[1] and Adam Biales[1], (1) U.S. Environmental Protection Agency, (2) Texas Tech University*

Neonicotinoid insecticides are some of the fastest growing and most commonly used pesticides globally. Imidacloprid (IMI), a primary neonicotinoid, has become a ubiquitous contaminant in surface waters near agricultural areas. IMI is effective in the prevention of insect infestation of crops but also poses potential adverse effects for non-target organisms, including invertebrate pollinators and aquatic vertebrates such as fish. A limited number of studies suggest oxidative stress and DNA damage may be associated with IMI exposure to fish. However, large-scale transcriptional or epigenetic analyses that could facilitate a better understanding of molecular mechanisms underlying these potentially toxic effects are lacking. In the current study we examine the effects of IMI during a critical developmental stage in the zebrafish (*Danio rerio*) model. Zebrafish larvae (n = 9-12 per group) were exposed from 4 hours post fertilization (hpf) through 5 days post fertilization (dpf) to an environmentally relevant dose of 100 ng/L (IMI-Low) and a higher dose of 1 mg/L (IMI-High) of IMI, a water-only control, and nicotine. Circadian rhythm behavioral tests, neurotransmitter measurements, DNA methylation and transcriptional changes were quantified to evaluate the risk IMI posed to developing zebrafish. Reduced Representation Bisulfite Sequencing (RRBS) identified 73 and 36 differentially methylated regions in genes for the low and high dose of IMI, respectively. RNAseq analysis identified 75 significantly expressed genes at the low IMI dose and 65 at the high IMI dose. Acetylcholine, GABA, L-Glutamine, and L-Glutamic acid levels were all significantly lower at IMI-High compared to control. Evidence for circadian rhythm dysregulation was found for larvae exposed to IMI-High. Results from this study suggests potential for IMI exposures to induce effects in aquatic invertebrates that include cell development perturbation, circadian rhythm disruption, and may be associated with neurological and cancer-promoting diseases.

**4.01.P-Th085 Application of fish tissue concentrations with effects from Tox21 high throughput screening assays in risk characterization**

***Ruth M. Sofield***[1]*, Maya Faber*[1] *and Christopher Andrew James*[2]*, (1) Western Washington University, (2) University of Washington*

High throughout cell-based and biochemical in vitro assays have recently been used to characterize risk in wildlife. In the Puget Sound, monitoring programs have established tissue concentrations in bivalves and several fish species. Our work is focused on how to compare the tissue concentrations to the in vitro assays in a risk characterization focused on contaminants of emerging concern in the Puget Sound.  Considerations have included whether whole body or specific organs are better measures of exposure and whether the Fish Plasma Model provides a more realistic exposure comparison to the in vitro assays.  In applying the exposure data to the risk characterization, we have also considered whether back-calculated water concentrations, calculated with BCFs from the tissue concentrations, are an appropriate comparison to the in vitro assays and to what extent chemical properties should be considered in the back-calculated water concentrations.  We will summarize our suggestions for best practices in using aquatic organism tissue concentrations with Tox21 high throughput screening assays.

**4.01.V Addressing Exposure and Risk Associated with Chemical Contaminants in the Era of Big Data**

**4.01.V-01 Accumulation-depuration data collection in support of toxicokinetic modelling**

***Sandrine Charles***[1] *and Aude Ratier*[2]*, (1) University Lyon, France, (2) Institut National de l'Environnement Industriel et des Risques (INERIS), France*

Regulatory bodies require bioaccumulation evaluation of chemicals within organisms to better assess toxic risks. toxicokinetic (TK) data are particularly useful in relating the chemical exposure to the accumulation and depuration processes happening within organisms. TK models are used to predict internal concentrations when experimental data are lacking or difficult to access, such as within target tissues. The bioaccumulative property of chemicals is quantified by metrics calculated from TK model parameters after fitting to data collected via bioaccumulation tests. In bioaccumulation tests, internal concentrations of chemicals are measured within organisms at regular time points during accumulation and depuration phases. the time course is captured by TK model parameters thus providing bioaccumulation metrics. But raw TK data remain difficult to access, most often provided within papers as plots. To increase availability of TK data, we developed an innovative database from data extracted in the scientific literature to support TK modelling. Freely available, our database can dynamically evolve thanks to any researcher interested in sharing data to be findable, accessible, interoperable, and reusable.

**4.01.V-03 Machine Learning as a Tool to Predict the Toxicity of Chemicals across Taxa**

***Jimeng Wu***[1,2]*, Christoph Schuer*[1]*, Simone D'Ambrosi*[3]*, Lilian Gasser*[4]*, Lorenz Ammann*[5]*, Julita Stadnicka-Michalak*[1]*, Fernando Perez-Cruz*[4]*, Kristin Schirmer*[1] *and Marco Baity-Jesi*[1]*, (1) Eawag - Swiss Federal Institute of Aquatic Science and Technology, Switzerland, (2) Paul Scherrer Institut, Switzerland, (3) Università di Roma La Sapienza, Italy, (4) Swiss Data Science Center, Switzerland, (5) Swiss Federal Research Institute WSL, Switzerland*

One of the pillars of modern civilization is the ability to synthesize and/or use an enormous range of chemicals, which allow for new and improved products and serve as pharmaceuticals, pesticides, food additives, and the like. However, in order to know that the use of these chemicals does not harm human- and ecosystem health, it is crucial to investigate the impact of these chemicals on those organisms potentially exposed. Usually, this is done through in-vivo testing. Yet, beyond its obvious ethical implications, in-vivo testing is not scalable to large

amounts of taxa and chemicals, because of its strong requirements in terms of time, money, and highly-trained personnel. Machine learning comes in as a viable alternative with the potential of allowing us to explore the impact of large numbers of chemicals on many taxa: harmlessly, quickly, and cheaply.
We develop machine learning models that can quickly infer the mortality of a chemical on a certain fish species and highlight the importance of including information on both chemical and taxonomy as input features. We then analyze ways of comparing the reproducibility of in-vivo experiments with that of machine learning models trained on similar data, highlighting that these comparisons are generally unreliable, and propose to upper bound the reproducibility of in-vivo experiments, instead of trying to exactly estimate it. Finally, we introduce an operational way of showing whether in-vivo mortality can be well-captured by in-vitro assays on fish-cell lines, finding evidence that it indeed can. Thus, machine learning and in-vitro assays present themselves as valid alternatives to animal testing, if we will be able to assess their limitations when utilized in out-of-domain settings (e.g. exotic chemicals and species).

## 4.02 Advances in Methodologies and Applications of Non-Targeted Analysis for PFAS

### 4.02.T-01 Communicating Confidence of Per- and Polyfluoroalkyl Substance Identification via High Resolution Mass Spectrometry

*Joeseph A. Charbonnet[1], Carrie A. McDonough[2], Feng Xiao[3], Trever Schwichtenberg[4], Dunping Cao[4], Sarit Kaserzon[5], Kevin Thomas[5], Pradeep Dewapriya[5], Benjamin Place[6], Emma Schymanski[7], Jennifer Field[4], Damian Helbling[8] and **Christopher Higgins**[9], (1) Iowa State University, (2) Stony Brook University, (3) University of North Dakota, (4) Oregon State University, (5) The University of Queensland, Australia, (6) National Institute of Standards and Technology (NIST), (7) University of Luxembourg, (8) Cornell University, (9) Colorado School of Mines*

Per- and polyfluoroalkyl substances (PFASs) are important environmental contaminants, yet relatively few analytical reference standards exist for this class. Nontarget analyses performed by means of high-resolution mass spectrometry (HRMS) are increasingly common for the discovery and identification of PFASs in environmental and biological samples. The certainty of PFAS identifications made via HRMS must be communicated through a reliable and harmonized approach. Here, we present a confidence scale along with identification criteria specific to suspect or nontarget analysis of PFASs by means of nontarget HRMS. Confidence levels range from level 1a—"Confirmed by Reference Standard," and level 1b—"Indistinguishable from Reference Standard," to level 5— "Exact Masses of Interest," which are identified by suspect screening or data filtering, two common forms of feature prioritization. This confidence scale is consistent with general criteria for communicating confidence in the identification of small organic molecules by HRMS (e.g., through a match to analytical reference standards, library MS/MS, and/or retention times), but incorporates the specific conventions and tools used in PFAS classification and analysis (e.g., the detection of homologous series and specific ranges of mass defects). Our scale clarifies the level of certainty in PFAS identification, and in doing so, facilitates more efficient identification

### 4.02.T-03 Environmental Forensic investigation of Chemical Manufacturing and Use of PFAS by Nontargeted Analysis

***James McCord**, U.S. Environmental Protection Agency*

Industrial producers and users of per- and polyfluorinated alkyl substances (PFAS) have been a major source of chemical contamination to nearby communities due to historical releases of legacy PFAS. Following the general phase-out of legacy PFAS such as PFOA/PFOS, industrial usage has shifted to alternative PFAS chemicals and it has been the domain of non-targeted analysis (NTA) to identify these replacement species. Environmental and public health organizations have a significant interest in identifying emerging chemical contaminants such as PFAS due to concerns for potential widespread release and the persistence and bioaccumulation of PFAS species. As such, over the last several years USEPA's Office of Research and Development (ORD) has applied non-targeted analytical approaches to the analysis of industrial effluent and contaminated environmental media (e.g. groundwater) provided by our state and regional partners to investigate the identity and quantities of legacy and emerging PFAS chemistries. Our NTA investigations using high-resolution mass spectrometry examined the chemical identities and abundances of PFAS in effluent and industrial use products. This research has identified multiple novel PFAS classes, including novel PFAS fluoroether species exhibiting ether linkages and acid head groups, chlorinated perfluoropolyethers (ClPFPECAs), and polyfluorinated side products of polyfluorovinylidine (PVDF). We also identified specific replacement chemicals used in applications such as metal plating and PFOS-free AFFF and have identified the presence of these replacement PFAS in local media, including biota. This presentation will discuss case studies of recent work by US EPA ORD in NTA investigations of PFAS sources. Examination of effluent-impacted media indicates that in the absence of treatment there is frequent presence of effluent-derived contaminants from industrial sources, even in the case of “non-contact” processes; source examination contains both intentionally added chemical species and production byproducts as common origins for emerging PFAS; and non-targeted interrogation of water treatment approaches for emerging contaminants indicates that existing PFAS treatment technologies can provide effective control for many undescribed PFAS species. The talk will additionally discuss efforts to ensure the consistent application of NTA approaches, transparent reporting of NTA results, and decision making using NTA site investigations.

**4.02.T-04 Hunting the missing fluorine: target, nontarget, and suspect screening of per-and polyfluoroalkyl substances (PFAS) in firefighting foams**

*Min Liu[1], Gabriel Munoz[2], **Caitlin Meara Glover**[1], Sung Vo Duy[2], Sébastien Sauvé[2] and Jinxia Liu[1], (1) McGill University, Canada, (2) University of Montreal, Canada*

Firefighting foams have been widely used for firefighter training and emergency responses. Among them, aqueous film-forming foams (AFFF) contain per- and polyfluoroalkyl substances (PFAS) as major components and therefore are major sources of environmental pollution by PFAS. The knowledge of PFAS compositions in AFFFs is necessary for contaminated site investigation and risk assessment. However, most of the published studies focused on the qualitative characterization of PFAS in AFFFs used in the US, while there remain great challenges in closing the fluorine mass balance. In the present study, we performed comprehensive characterizations of PFAS in 25 foams (21 PFAS-containing foams, 2 fluorine-free foams, and 2 non-AFFFs) from non-US markets made between 1980 and 2016. Target and nontarget analyses and suspect screening were realized using Orbitrap high-resolution mass spectrometry (HRMS). Validated total oxidizable precursor (TOP) assay and total organofluorine analysis (TOF) using combustion ion chromatography (CIC) were also carried out. The results confirmed that the two fluorine-free AFFFs and two non-AFFFs contain little or no PFAS or fluorine, while the identified PFAS in the PFAS-containing AFFFs span over a wide concentration range (5973~120944 mg/L). Non-targeted analysis revealed multiple novel classes of polyfluoroalkyl substances, including 9 classes of electrochemical fluorination (ECF)-based PFAS, 9 classes of fluorotelomer (FT)-based PFAS and 2 other classes. The TOP assay also revealed a significant fraction of unknown precursors in ECF AFFFs (17.9~72.9 mol%) and 4 out of 14 FT AFFFs (10.8~47.4 mol%), while the precursors in the other 10 FT AFFFs can be largely captured by HRMS. The high amount of unknown fluorine in ECF AFFFs was also confirmed by the CIC. Good fluorine mass balance was achieved for the FT AFFFs by comparing total fluorine with the molar sums of fluorine from the PFAS obtained by HRMS.

**4.02.T-05 IR-MALDESI Imaging of Per and Polyfluoroalkyl Substances (PFAS) in Stabilized Soil Cores**

***Theresa Guillette**[1], Whitney Stutts[2], Andy Baumeister[1], David Liles[1], Johnsie Lang[1] and Theresa Olechiw[1], (1) Arcadis U.S., Inc., (2) North Carolina State University*

*In-situ* stabilization and solidification (S/S) is a recognized source zone technology to reduce mobilization of numerous constituents in impacted soils. Previous research has shown that soil stabilization decreased per and polyfluoroalkyl substances (PFAS) leachability over a period of 2 years at an aqueous film forming foam (AFFF) source zone. While this supports reduced PFAS leachability using S/S, mechanisms on how stabilization occurs within *in-situ* treatment is unknown. The objective of this project was 1) develop a mass spectrometry-based imaging technique for PFAS on stabilized and control soil cores to understand potential mechanisms for soil stabilization and 2) determine if chemical features of the stabilizing agents were associated with PFAS in soil cores. Infrared matrix-assisted laser desorption electrospray ionization (IR-MALDESI) was coupled with high resolution, accurate-mass mass spectrometry (HRAM-MS) to image PFAS in soil cores from an AFFF-impacted source. Baseline soil characterization at the site indicated that 98.2% of the total analyzed PFAS consisted of perflurohexane sulfonic acid (PFHxS), perfluorosulfonic acid (PFOS), perfluorohexanoic acid (PFHxA), and perfluorooctanoic acid (PFOA). PFOS had the highest concentrations in soil and ranged from 0.8 µg/kg to 1,760 µg/kg. Stabilized and control soil cores were kept intact, frozen at -20°C, then cut with an oscillating saw at a cross-section to ensure the same thickness across the entirety of the section. Soil cores were imaged in negative ion mode and *m/z* range of 100-1000 Da. Targeted and non-targeted analysis were conducted; features were identified using suspect lists and non-targeted analysis were presented as raw abundances matched against several databases. For the targeted approach, neat standards (CIL ES-5639) were used to develop the method and IR-MALDESI detected between 0.1 pg - 2 ng on target for several PFAS analytes. There were distinct differences between controls and stabilized soil cores for PFOS and PFHxS, with higher abundance found in stabilized soil cores. Preliminary non-targeted analysis revealed 120 distinct features in the stabilized soil cores compared to the control soil cores, with identification of several PFAS precursors (PFOSA, N-Propylperfluorooctanesulfonamide). Ongoing analysis will determine if certain chemical characteristics of baseline stabilizing agents associate with PFAS in stabilized soil core sections.

**4.02.T-06 Reconstructing Temporal PFAS Trends from a Sediment Core with Targeted and Nontargeted Analysis**

***Michaela Cashman**[1], Anna Robuck[2], Maya Morales-McDevitt[3], Jeremy Koelmel[4], Thomas Boving[3] and Mark Cantwell[1], (1) U.S. Environmental Protection Agency, (2) Icahn School of Medicine at Mount Sinai, (3) University of Rhode Island, (4) Yale University*

Use of Per- and polyfluoroalkyl substances (PFAS) in commercial and consumer products predates techniques for their identification in environmental matrices. One approach to determine past PFAS releases to the environment is through examining radiometrically dated sediment cores. In this study, a sediment core was collected from the Pawtuxet River in Rhode Island, USA. The dated sediment core was analyzed for PFAS to develop an age-depth model of PFAS deposition. The coring site was a depositional area near former textile mills suspected to have used PFAS containing products. Initial targeted analysis showed 18 unique PFAS compounds in the core with long-chain perfluoroalkyl carboxylic acids (PFCAs) dominating the core profile. Peak ∑PFAS concentrations of 55 ng/g were seen in 1973. A modified total oxidizable precursor (TOP) assay and extractable organic fluorine (EOF) extraction indicated fluorine detected through targeted analysis accounted for <6% of fluorine detected in EOF, with peak fluorine detections occurring at 1976 and at the core surface (2021). Data from nontargeted liquid chromatography high-resolution mass spectrometry analysis were evaluated using FluoroMatch software. Over 20 additional PFAS compounds, including unsaturated and H-substituted PFCAs were determined. The additional compounds identified in nontarget analysis explain the unaccounted fluorine from EOF and further constrain temporal releases and distribution of PFAS from former manufacturing facilities. Ongoing research uses these data to track the transport of contaminated sediments from legacy sites in the aquatic environment.

**4.02.T-07 Evaluations of in vivo and in vitro Dosimetry and Metabolic Biotransformation of the Hexafluoropropylene Oxide Homologue HFPO-TeA, an Emerging Per- and Polyfluoroalkyl Substance (PFAS)**

***Denise MacMillan**[1], Aero Renyer[2], Marci Smeltz[1], Michael DeVito[1], Michael Hughes[1], Leah Wehmas[1] and Barbara A. Wetmore[1], (1) U.S. Environmental Protection Agency, (2) Oak Ridge Institute for Science and Education (ORISE)*

The prevalence, persistence, and potential adverse health effects of legacy per- and polyfluoroalkyl substances (PFAS) led to government restrictions, voluntary phase outs, and the need for replacements with similarly useful but less toxic properties. Emerging PFAS such as hexafluoropropylene oxide (HFPO) homologues are used in fluoropolymer manufacture as alternatives to perfluorooctanoic acid. Recent detection of perfluoro-2,5,8-trimethyl-3,6,9-trioxadodecanoic acid (HFPO-TeA) in surface waters combined with the dearth of hazard data creates potential for exposure with unknown health effects. To address this issue, the US Environmental Protection Agency conducted a 5-day, repeat dose-response study in male and female Sprague-Dawley rats to evaluate *in vivo* hazard, dosimetry, and HFPO-TeA biotransformation products (BTPs). After daily administration of HFPO-TeA at doses ranging from 0.3 to 17 mg/kg/day, in-life observations consisted of weight loss and abnormal breathing with potential sex-dependent differences. Female rats lost weight starting at the 6.3 mg/kg/day dose; weight loss for males did not occur until the 17 mg/kg/day dose. Plasma, liver, and kidney were collected to evaluate internal dose and target tissue disposition, and probe for BTPs. Targeted analysis of plasma collected 24 hr after the $5^{th}$ dose revealed significantly higher HFPO-TeA in female rats than males at doses of 2.3 mg/kg/day and above. Non-targeted analysis (NTA) revealed potential BTPs of HFPO-TeA, including perfluoro-2,5-trimethyl-3,6-dioxanonanoic acid (HFPO-TA), which was present in plasma of both sexes, but at a lower abundance in females. Data visualization showed separation of exposed plasma vs. controls, and clustering according to sex. Cross-species and cross-sex evaluations of *in vitro* hepatocyte clearance and plasma protein binding are underway to further characterize sex and species differences and conduct *in vitro-in vivo* comparisons of HFPO-TeA internal dosimetry estimation and biotransformation characterization. Determinations of HFPO-TeA and BTP levels in liver and kidney are also underway to round out evaluations of target tissue dosimetry and metabolic biotransformation. These data are improving our understanding of HFPO homologue biotransformation and disposition, enabling a more robust risk-based evaluation of a key emerging PFAS group. Disclaimer: This abstract does not necessarily represent the views or policies of the US Environmental Protection Agency.

**4.02.T-08 Non-Targeted mapping of PFAS in drinking water using high-resolution mass spectrometry combined with retention time prediction**

*Anca Baesu, **Yong-Lai Feng** and Yan Li, Health Canada*

Per- and polyfluoroalkyl substances (PFAS) are a class of thousands of synthetic chemicals, many of which are used worldwide to make products resistant to water, heat, and stains since the 1950s. Some PFAS do not break down easily and therefore stay in the environment for a very long time, especially in water. Studies show that exposure to even trace amounts of some PFAS is linked to harmful health effects. PFAS is not regularly monitored at water treatment plants in Canada and the USA. The health advisory level established by the EPA for two PFAS (PFOA and PFOS) combined concentration in drinking water is 70 ppt. The current monitoring program in Canada only targets the most commonly researched PFAS and the number of PFAS characterized in exposure assessments is still very small compared to PFAS registered in the market let alone transformation products and metabolites in the environment. This study developed a non-targeted analysis method that can map a large number of PFAS in drinking water and source water quickly using high-resolution mass spectrometry. A retention time prediction model was also developed for use in combination with a rapid PFAS diagnostic ion search tool to increase confidence in PFAS identifications. The results showed that the developed NTA method was able to map the difference between treated water and the source water to evaluate the efficiency of water treatment for PFAS removal and drinking water quality.

**4.02.P Advances in Methodologies and Applications of Non-Targeted Analysis for PFAS**

**4.02.P-Mo088 Non-Targeted Analysis of Per- and Polyfluoroalkyl Substances Found in AFFF Impacted Surface and Groundwater Measured by Passive Samplers**

***Riley Hershberger** and Paul Edmiston, College of Wooster*

Passive sampling is being evaluated for the time-integrative measurement of per- and polyfluoroalkyl substances (PFAS) in water. A key question is whether passive samplers provide an accurate and comprehensive of measurement of aqueous phase PFAS concentrations in water regardless of chemical structure. Paired grab samples and passive samplers were deployed at Ellsworth and Peterson Air Force Bases having either surface water and groundwater impacted by AFFF, respectively. In addition to a targeted approach using HPLC-MS/MS (QQQ), a non-targeted approach was employed using high resolution mass spectrometry HPLC-Q/ToF in negative ion mode. PFAS analytes were identified via suspect screening using the NIST library followed by MS/MS fragment analysis and library matching. Surrogates were used for quantitative and semi-quantitative measurement of PFAS concentration. An unbiased approach using mass defect to identify unknown PFAS was also used to screen the data. Results showed that passive samplers have lower sampling rates for shorter chain PFAS analytes such as perfluorobutanoic acid. In addition to surface and groundwater, an initial non-targeted analysis of PFAS in stormwater will be reported.

**4.02.P-Mo089 Development of an Open-Source Mass Spectral Database for the Identification of Per- and Polyfluoroalkyl Substances**

***Benjamin Place*** *and Jared Ragland, National Institute of Standards and Technology (NIST)*

Identification of per- and polyfluoroalkyl substances (PFAS) in environmental matrices without the use of analytical standards requires the application of non-targeted analysis (NTA) techniques, typically performed with high resolution mass spectrometry. Confident, probable identifications of PFAS often require reference mass spectra produced from known PFAS standards. Unfortunately, the lack of analytical standards for most PFAS limits the ability to produce quality reference mass spectra. Research laboratories instead produce reference libraries of mass spectra using chemical mixtures (or even environmental samples) and store these data in platform dependent, often proprietary, formats. Researchers at the National Institute of Standards & Technology have developed a database infrastructure and associated data tools to extract, store, and analyze mass spectral data related to PFAS in both simple (analytical standards) and complex (environmental samples) mixtures. The approach is aimed at leveraging the wealth of data generated by PFAS research laboratories through their previous studies. Systematic collection of metadata, including sample, analytical method, and compound annotation information, allows for the contextualization of the quality of each mass spectrum. Users of the database will be able to query their unknown mass spectra against the library and, using the metadata and uncertainty analysis, discover potential PFAS identities to determine the confidence of their identifications. The design of the database and its functionality, using real environmental samples, will be presented.

**4.02.P-Mo090 Per/Polyfluoroalkyl Substances (PFASs) in White Shark Serum and Muscle Using HRMS Screening Techniques**

***Jennifer M. Marciano****, Lisa M. Crawford, Anne E. McElroy and Carrie A McDonough, Stony Brook University*

As top marine predators, white sharks (*Carcharodon carcharias*) are at risk for toxicity from persistent contaminants through biomagnification. Per/polyfluoroalkyl substances (PFASs) are a class of persistent and toxic organic contaminants that are widespread in the marine environment. Serum (N=27) and muscle (N=18) samples from white sharks were obtained by the non-profit research organization OCEARCH from various coastal locations in the North Atlantic Ocean, ranging from Sydney, Nova Scotia to Jacksonville, Florida. Serum samples were processed using a high-throughput protein crash and lipid removal approach and analyzed by liquid chromatography and quadrupole time-of–flight mass spectrometry (LC-QTOF-MS) for quantitative targeted and qualitative nontarget analysis. Serum samples were analyzed by targeted quantitation to measure 47 PFASs, including 13 perfluorocarboxylates (PFCAs) and 12 linear, branched, and cyclic perfluoroalkyl sulfonates (PFSAs), and several sulfonamide and fluorotelomer precursors. Limits of quantitation were at or below 0.1 ng/mL for most compounds and average spike recoveries ranged from 69 to 123%. Long-chain (C10-C13) PFCAs and the C8 (PFOS) and C10 PFSAs were frequently detected. The C12 PFCA (PFTrDA) was generally most abundant among targeted PFASs at concentrations ranging from 0.2 – 0.9 ng/mL. Samples were also screened for a much larger list of known and novel PFASs using Agilent Profinder software. HRMS screening revealed widespread occurrence of additional long-chain PFCAs (e.g., PFPeDA), for which no analytical standards are available, and several frequently detected peaks tentatively identified as polyfluorinated PFASs or metabolic conjugates. Serum PFAS profiles will be compared to profiles from muscle tissue to improve our understanding of PFAS tissue distributions in white sharks. This study will provide novel insight into the fate of PFASs in this vulnerable apex predator.

**4.02.P-Mo091 Use of electron activated dissociation (EAD) on the ZenoTOF 7600 system to elucidate PFAS structures**

***Karl Oetjen***, *Craig M. Butt, Megumi Shimizu and Diana Tran, SCIEX*

Poly- and perfluoroalkyl substances (PFAS) are well-known environmental contaminants and are widely detected in humans and wildlife, water, soil and air. PFAS are primarily used for their stain repellency properties as well as their surfactant characteristics, for example, in foams to combat petroleum fires. Even though there are an estimated 5,000 unique PFAS industrially manufactured, most monitoring efforts are focused on only 20-30 compounds. Non-target acquisition using high resolution accurate mass spectrometry is beneficial for elucidating unknown compound structures, such as PFAS. However, traditional fragmentation methods using collision-induced dissociation (CID) can be too aggressive to form diagnostic MS/MS spectra. Alternatively, electron activated dissociation (EAD) has shown potential as a form of fragmentation.

Standard solutions of 5 PFAS compounds (5:3 FTB, 5:1:2 FTB, AmPr-FHxSA, TAmPR-FHxSA, 6:2 FTSA-PrB), were infused on the ZenoTOF 7600 system using both CID and EAD fragmentation modes. In separate EAD experiments, the kinetic energy (KE) was ramped from -10 to 25 V and the electron beam current ramped from 0 to 8000 V. Further, 10, 35 and 100 ms reaction times were tested.

EAD generated a more comprehensive MS/MS fragmentation spectrum compared to CID, which resulted in additional structural information for improved compound elucidation. Preliminary tests were focused on the 5:1:2 fluorotelomer betaine in positive electrospray ionization mode. Using CID fragmentation, the only fragment formed was the $[C_3H_8N]^+$ ion at m/z 58.065 Da. The SCIEX Fluorochemical HR-MS/MS Spectral Library 2.0 contains the fragmentation spectra for several fluorotelomer betaines and confirms that the m/z 58.065 Da ion is the only significant fragment formed with CID. In contrast, the EAD fragmentation spectrum showed many fragment ions that corresponded to the unzipping of the carbon backbone of the molecule. For example, sequential losses of $CF_2$ were observed. The presence of additional fragment ions in the more comprehensive MS/MS spectrum generated by EAD is more diagnostic of the unique fluorotelomer betaine compounds and therefore can provide more structural information.

**4.02.P-Mo093 Comparison of Acquisition Modes for Targeted & Non-Targeted analysis of PFAS by LC-HRMS**

*Tarun Anumol[1], James Pyke[1], Emily Parry[1], Ralph Hindle[2], Kathy Hunt[2], Bradley Clarke[3] and* ***Samuel Haddad***[1]*, (1) Agilent Technologies, Inc., (2) Vogon Labs, Canada, (3) The University of Melbourne, Australia*

Per- and polyfluorinated alkyl substances (PFAS) are ubiquitous in the environment, with thousands of compounds identified in this chemical classification. When the use of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) were replaced, industry developed many other compounds that were unregulated, increasing the complexity of identification and quantitation. Liquid chromatography with triple quadrupole (LC/TQ) mass spectrometry (MS) is the gold standard for sensitivity and highly selective quantitative analysis of PFOS, PFOA, and numerous other PFAS where commercial analytical reference materials are available, but the data cannot be used for additional compounds that were unknown at the time of analysis. Quadrupole time-of-flight (QTOF) MS provides high resolution accurate mass (HRAM) data that provides similar sensitivity to TQ, wide linearity ranges, accurate mass specificity, and can be retrospectively searched for additional compounds not included in an initial targeted list. QTOF data can be collected in a choice of acquisition modes to provide not only precursor ion detection, but also successively more specific modes that aid in determining product ions that can be used for structural elucidation of previously unknown PFAS. This study systematically compared quantitative performance for 25 PFAS in a number of data acquisition modes including targeted MS/MS (data dependant acquisition) and All Ions and Q-RAI (data independent acquisitions) on an LC-Q/TOF and MRM mode on an LC/TQ. Comparison metrics included limits of detection (LOD), linear dynamic range (LDR), accuracy and precision of replicates along with other 'soft' factors like data processing speed, file size, ability to perform suspect and unknown screening and others. This work is the first known to make such a comparison for PFAS quantification using same chromatography and multiple acquisition modes using a TQ and QTOF-HRMS.

**4.02.P-Mo094 Per- and Polyfluorinated Alkyl Compound (PFAS) Analysis in Cosmetics Using High Resolution Accurate Mass Spectrometry**

***Craig M. Butt***[1]*, Mikyanny Reyes*[1]*, Holly Lee*[1]*, Keegan Harris*[2] *and Amy Rand*[2]*, (1) SCIEX, Canada, (2) Carleton University, Canada*

Per- and polyfluorinated alkyl compounds (PFAS) have been detected in a wide range of cosmetics and personal care products. PFAS-containing cosmetics may act as human exposure sources through either dermal absorption or ingestion. Previous studies have shown that a large portion of the total fluorine in cosmetics is unaccounted for using traditional, targeted analysis techniques. Therefore, there is a need for non-targeted acquisition methods to comprehensively identify PFAS compounds in cosmetic samples. In this study we analyzed a series of cosmetic extracts on the SCIEX ZenoTOF 7600 system using non-targeted acquisition. Commercially available cosmetic samples were extracted with methanol using sonication and cleaned using ENVI-Carb SPE cartridges. Information dependent acquisition (IDA) was performed using the Zeno Trap to improve duty cycle thereby producing stronger MS/MS spectra for improved compound identification. Data were initially processed using suspect screening with MS/MS library matching for compound confirmation. A diverse range of PFAS were detected including perfluorinated carboxylic acids, mono- and di-alkyl fluorotelomer phosphates and fluorotelomer sulfonates. In addition, samples were interrogated using the peak finding algorithm in the SCIEX OS software. Additional PFAS were detected, including several PFAS compounds that have not been reported, to our knowledge.

**4.02.V Advances in Methodologies and Applications of Non-Targeted Analysis for PFAS**

**4.02.V-01 Non-targeted Analysis (NTA) for the Screening of Per- and Polyfluoroalkyl Substances in Drinking and Surface Water Samples from South Florida Environments.**

***Xuerong Li****, Danni Cui, Brian Ng, Piero Gardinali and Natalia Soares Quinete, Florida International University*

Per- and polyfluoroalkyl substances (PFAS) are a group of anthropogenic pollutants that are found ubiquitously present in surface and drinking water supply. It poses big concerns on human and environmental exposure, considering their persistent feature, bioaccumulative potential, and significant adverse health effects at low concentrations. Non-targeted analysis (NTA) approaches are being increasingly used in the identification of thousands of unknown PFAS, including their precursors, degradants, and metabolites, which are not regularly monitored by current target analysis due to the lack of analytical standards and scientific knowledge on fate and transformation of these contaminants.

In this study we have developed an NTA workflow based on an online solid phase extraction (SPE, using a Water WAX column)- liquid chromatography (LC)- high-resolution mass spectrometry (HRMS) method using a Q-Exactive Orbitrap system for the screening of PFAS species in drinking waters from the most populated counties, as well as in surface waters from Biscayne Bay canals, Key West, and Everglades canals, in South Florida. Water samples were run in full scan negative mode with a scan range from 100.0 to 800.0 m/z at a resolution of 140,000, followed by data dependent MS/MS with a normalized collision energy of 30 and at a resolution of 35,000. Data post-processing workflow was conducted and optimized using Compound Discoverer 3.3 and FluoroMatch 2.6 with specific criteria proposed for data reduction and higher confidence of identification, such as blank filtering, mass defect filtering, class scoring for common fragments, and fragmentation match in EPA PFAS Master list. In total, over 500 PFAS were tentatively identified from the two data processing workflows, with little overlap. Major PFAS species, such as chlorinated PFAS, 7-(heptafluoropropyl)-4,9-dimethoxy-5H-furo[3,2-g][1]benzopyran-5-one, 2-[Chloro(difluoro)methoxy]-1,1,2,2-tetrafluoroethanesulfonic acid, 5-(1,1,2,2,3,3,4,4,4-nonafluorobutyl)-1H-pyrimidine-2,4-dione, and 3,3,3',3'-tetrakis(trifluoromethyl)-1,1'-spirobi[2,1-benzoxaphosphol-1-ium] were tentatively identified in different water types. A Semi-quantitation method to estimate concentration for PFAS identified by NTA will be proposed based on a calibration curve built from the averaged response factor of a mixture of native standards and internal standards.

## 4.03 Airborne and Volatile Per- and Polyfluoroalkyl Substances: Measurements and Recent Developments

### 4.03.T-01 Introductory Remarks - Airborne and Volatile Per- and Polyfluoroalkyl Substances: Measurements and Recent Developments

***Alix E. Rodowa***[1]*, Jessica Lynn Reiner*[1] *and Cora Young*[2]*, (1) National Institute of Standards and Technology (NIST), (2) York University, Canada*

### 4.03.T-02 Chemical ionization mass spectrometry for online gas and aerosol-phase per- and polyfluoroalkyl substances (PFAS) analysis using iodide reagent ion

*Bailey Bowers*[1]*,* ***Ryan C. Sullivan***[1] *and Joel Thornton*[2]*, (1) Carnegie Mellon University, (2) University of Washington*

Per- and polyfluoroalkyl substances (PFAS) are a class of ultra-persistent, toxic, anthropogenic contaminants. PFAS are ubiquitous in environmental and built systems, but very few online methods exist for their characterization in atmospheric gasses and aerosols. Iodide time-of-flight chemical ionization mass spectrometry (iodide-ToF-CIMS) is a promising approach for online characterization of PFAS in the atmosphere. Previous work using iodide-ToF-CIMS was successful in measuring gas-phase perfluorocarboxylic acids and fluorotelomer alcohols, but those are just two of the myriad PFAS that are atmospherically relevant. Therefore, our first objective was to test other sample introduction methods coupled to iodide-TOF-CIMS to evaluate their ability to measure a wider suite of PFAS in both gas and aerosol phases. Using a variety of sample introduction techniques, we successfully measured gas-phase fluorotelomer alcohols (FTOH), gas and aerosol phase perfluorocarboxylic acids (PFCA), and aerosol phase perfluorosulfonic acids and polyfluoroalkyl phosphoric acid diesters (PFSA and diPAP). Furthermore, despite their ubiquity, PFAS are a poorly understood class of molecules that exhibit unusual and often unexpected physicochemical properties due to their highly fluorinated nature. Since detection of PFAS with iodide-ToF-CIMS relies on the PFAS molecule to either undergo proton transfer or adduct formation with iodide, understanding PFAS behavior during chemical ionization gives rise to a more fundamental understanding of these compounds. Through voltage scanning experiments and DFT calculations, we found that PFCA and FTOH readily form iodide adducts, while PFSA and diPAP preferentially undergo proton transfer with iodide. Generally, binding energy increased with increasing linear chain length, and PFCA had stronger binding than FTOH. We also evaluated the detection limits and sensitivity factors for some PFAS substrates. Overall, our results suggest that iodide-ToF-CIMS can be used to measure even nonvolatile PFAS such as PFSA and diPAP in the aerosol phase in a real-time, online fashion.

### 4.03.T-03 Volatile Per- and Polyfluoroalkyl Substances in Air: Developing Methods for Targeted Analysis and Discovery of Non-Target Species

***Nathan Shafer***[1]*, Hannah Calder*[1]*, Tarun Anumol*[2]*, Ericka Hachmeister*[1]*, Laura Miles*[1] *and Carlos Gil*[1]*, (1) Markes International (2) Agilent Technologies, Inc.*

Per- and polyfluorinated alkyl substances (PFAS) are analysed mainly by liquid chromatography, as traditionally the focus has been on monitoring PFAS in soil and water, targeting mainly the ionic PFAS species. Nevertheless, trace PFAS quantities in air are also of great concern from human health and environment. The analytical technology required by air monitoring scientists to address this area is already available and tested but has not been utilised widely for PFAS measurements up to this point.

Modern analytical TD–GC–MS systems were designed specifically for monitoring trace-level organic vapours and recent developments in automated Thermal Desorption (TD) technology have meant these methods can be applied to more and more challenging compounds. With the wide range of different chemistries as displayed within the classes of PFAS compounds, TD technology can be implemented to monitor PFAS in air. The aim of this study was to evaluate the performance of the latest off-the-shelf sorbent tube sampling and automated TD–GC–MS analytical technology for analysing volatile and semi-volatile PFAS.

This study focused on perfluoroalkyl carboxylic acids (PFCAs - C4 to C14), fluorotelomer alcohols (FTOHs), fluorotelomer carboxylic acids (FTCAs) and fluorotelomer sulfonamides (FOSAs). These compounds have previously been shown to be widely distributed within air, because of their high volatility and ease of transport.

The fact that very volatile perfluorinated hydrocarbons (C1 to C3) are part of PFAS family is often overlook, but these are also potent greenhouse gases and ozone depleting substances. These species require whole-air sampling using canisters, sampling bags or online monitoring but have the potential to be included within the TD analytical system.

Method development was carried out in the same manner as for all VOCs analysis. Guidelines from established methods, like the US EPA TO-17, can be directly transferred into this type of analysis. As single quadrupole MS with EI are often used for standard air monitoring methodology, this detector was chosen for the project, however it should be noted that other detectors may improve the sensitivity and aid in finding PFAS amongst the background of other components. Excellent method performance (linearity, repeatability in the order of 5% or less, storage stability tested up to 15 days, etc.) was demonstrated across the range of compounds tested, including low or sub-ppt detection limits for all compounds.

**4.03.T-04 Analysis of trace-level volatile and emerging PFAS derived from Biosolids using GC Quadrupole Time of Flight HRMS**

*Tarun Anumol, Sofia Nieto and* ***Samuel Haddad****, Agilent Technologies, Inc.*

Persistent organic pollutants (POPs) like Dioxins, Furans and Chlorinated paraffins (CPs) are regulated and restricted from production globally as declared by the UN Stockholm Convention. Recently, some per- and polyfluoroalkyl substances (PFAS) were added to the list of POPs too. PFAS are a class of several thousand fluorinated contaminants that are created intentionally for anthropogenic use. The analysis of many PFAS occurs by LC/MS but recently studies have indicated a large portion of PFAS may be volatile and semi-volatile that escapes into the air. These kinds of PFAS are more suited to GC/MS analysis and could be missed by LC/MS leading to under-representation in the mass balance. While GC single quadrupole & triple quadrupole mass spectrometers are great tools for the quantitative analysis of targeted PFAS classes like the fluorotelomer alcohols and other shorter chain acids, this is dependent on availability of analytical standards which is limited. Further, we have noticed potential for matrix interferants and isobaric PFAS to cause false positives or misreporting of specific PFAS in air and biosolids. This study used a GC coupled to high-resolution quadrupole time-of-flight mass spectrometer to develop an analytical method for quantification of fluorotelomer alcohols and other shorter chain PFAS while also performing suspect screening using the NIST library on biosolid samples. Finally, we evaluated the samples for unknown PFAS and making use of the QTOF, structural identification and putative ID was done. Examples of structure elucidation of PFAS and distinction of different class of volatile PFAS in biosolids were found using the HRMS. This study shows the complementary nature of GC-HRMS to LC/MS techniques for analysis of emerging PFAS to close the mass balance in environmental studies.

**4.03.T-05 High Time Resolution Ambient Measurements of Gas- and Particle-Phase Perfluorocarboxylic Acids (PFCAs): Implications for Sources and Fate**

***Cora Young***[1]*, Shira Joudan*[1]*, Jessica Clouthier*[1]*, Trevor VandenBoer*[1]*, Jeremy Wentzell*[2] *and John Liggio*[2]*, (1) York University, Canada, (2) Environment and Climate Change Canada*

Per- and polyfluoroalkyl substances (PFAS) are widely used in numerous consumer and industrial products, including stain repellents, non-stick materials, and firefighting foams. A class of PFAS, perfluorocarboxylic acids (PFCAs), are found ubiquitously in the environment, including in remote regions far from sources. While PFCAs can be directly emitted into the atmosphere through production and use of fluoropolymers and consumer products, they can also be formed by atmospheric oxidation of volatile PFAS. A complete understanding of the sources, fate, and transport requires atmospheric PFCA measurements. Current atmospheric sampling methods for PFCAs primarily rely on offline sampling techniques, such as measurements using annular denuders, sorbents, and/or filter packs with collection timescales of several hours to days. Atmospheric processes (e.g. atmospheric oxidation, gas-particle partitioning) that drive production and fate of PFCAs occur on faster timescales. As a result, measurements on long timescales offer limited insight into these processes. Faster analyses on the timescale of seconds to one hour can give extremely valuable information on the temporal trends and concentrations of analytes, allowing relationships to be conclusively made with other atmospheric parameters (e.g. meteorology). This remains a major gap in our understanding of PFCA in the atmosphere. Here, we describe two new approaches to high time resolution PFCA atmospheric measurements, as well as their application to ambient measurements in Toronto, Canada. The ambient ion monitor-ion chromatograph-mass spectrometer (AIM-IC-MS) provides in situ hourly measurements of both gas- and particle-phase PFCAs. The time-of-flight chemical ionization mass spectrometer (ToF-CIMS) measures in situ gaseous PFCAs at a timescale of 10 seconds. Data from the AIM-IC-MS and ToF-CIMS, along with co-located meteorological and air quality parameters will be used to describe new insights into PFCA sources, fate, and transport.

**4.03.T-06 Analysis of PFAS Compounds in Indoor Air using Thermal Desorption GC-MS in Retrospective: Frustrations, Successes, and Three Years of Progress**

***Kurt Thaxton***[1]*, John R. Stuff*[1]*, Megan C. Harper*[1]*, Tarun Anumol*[2] *and Jesse Miller*[3]*, (1)GERSTEL, (2) Agilent Technologies, Inc., (3) CAMSCO*

The fate of PFAS in air, both in the outdoor and indoor environments, is relatively unknown and is a new area of concern. Current PFAS measurement techniques involve capturing PFAS onto XAD or similar resins and extracting them with solvents; the extracts are then run in a way similar to those in drinking water. However, the analysis of extracts results in relatively poor analysis sensitivity due to the loss of analytes in the bulk of the un-analyzed extract.

GERSTEL, in partnership with Eurofins Air Toxics, Agilent Technologies, and CAMSCO have described the use of thermal desorption, gas chromatography, and tandem mass spectrometry (TD-GC-MS/MS) as means to perform demanding PFAS in air work where the matrix is challenging due to high volume sampling, or due to issues with a complex environmental matrix (vapor intrusion, etc.). The work has been successful, with many academic presentations at conferences, commercial sampling and analysis of PFAS under way, and the contribution of this work to two PFAS standards in development at ASTM.

However, over the past three years of method development, all involved have been surprised by the issues surrounding the analysis of PFAS species in air. For example, these compounds, which can have molecular weights in the 500 amu range, elute rapidly from strong columns and at retention times that are similar to VVOC's such as propylene (42 amu nominal). Perfluorinated carboxylic acids, which should be problematic due to the acid functionality, generally tail less than alcohols. And of course, the ever-present PTFE/Teflon/PFAS background during sampling and within instruments remains an issue for both false positives and negatives, as it has been in drinking water analysis by LC-MS.

This talk will be a retrospective of three years of work regarding the selection of relevant PFAS species for both indoor air and vapor intrusion, sampling and choices of sampling media, and gas chromatographic issues with a particular focus on column selection. In addition, detection strategies will also be discussed. Although the original work focused on using single quadrupole MS detectors, the work progressed into the need for a triple quadrupole MS to deal with the matrix issue surrounding high volume sampling. Although the best choice, even then, sometimes a fluorine-specific detector, such as an ECD, may be helpful.

**4.03.T-07 Clearing the Air on Per- and Polyfluoroalkyl Substances (PFAS) in AC Filters**

***John A. Bowden**, Alina Timshina and Willy Sobczak, University of Florida*

Mounting evidence has shown that per- and polyfluoroalkyl substances (PFAS) are ubiquitously present in our living environment, as they have been detected in homes, office spaces, and even daycare facilities. While the sources of these personal exposures are varied (e.g., carpets, textiles, food products, furniture, to name a few), one interesting direction, supported by several recent studies, is to examine dust as a potential composite matrix for assessing PFAS burden in living environments. Here, we have investigated the utilization of disposable air condition (AC) filters as a potential sample collection strategy that is simple, cost effective and scalable to examining large cohorts of households. We examined three sub-categories of AC filters (homes, vehicles, campus buildings), with corresponding clean (unused) filters tested as blanks for each brand/type tested. Each filter was examined in triplicate, with three randomly selected swatches selected for extraction. The AC filters were examined using a robust methanol rotation/sonication extraction combined with isotope dilution and a targeted liquid chromatography – tandem mass spectrometry (LC-MS/MS) method (Thermo Scientific Vanquish and a TSQ Quantis triple quadrupole MS) that monitors over 90 PFAS. While several different PFAS were detected in the AC filters, interestingly, the most abundant species were polyfluorinated dialkylated phosphate esters (diPAPs). Further, a subset of the examined filters were also extracted and analyzed using an Orbitrap Exploris gas chromatograph (GC) 240 for the high resolution accurate mass analysis of volatile PFAS. The presentation will highlight the varied detection of PFAS across the different filter types and categories, along with a discussion on the feasibility of using AC filters for large scale household testing.

**4.03.T-08 Source-Receptor Relationships for Atmospheric PFAS Determined Using a 3-D Atmospheric Chemical Transport Model and Spatially Resolved US Emissions**

***Jennifer Sun**[1], Colin Thackray[1], Joy T. Morgan[2] and Elsie Sunderland[1], (1) Harvard University, (2) Michigan Department of Environment, Great Lakes, and Energy*

Atmospheric emissions of PFAS are increasingly recognized as an important source of drinking water and soil contamination next to point sources yet are relatively understudied. Volatile precursors are oxidized in the atmosphere to terminal PFAS, although complete understanding of transformation pathways is lacking. Here we build upon an existing global model for fluorotelomer precursor transport and deposition of terminal perfluorocarboxylic acids using a 3-D global atmospheric transport model (GEOS-Chem). We present a new nested simulation that enables the development of higher-resolution (0.5°x0.67°) source-receptor relationships for atmospheric PFAS in the continental US. Key model updates include the addition of perfluorosulfonic acid (PFSA) formation, deposition from sea spray aerosols, and PFAA particulate partitioning. We spatially distribute emissions by source sector using publicly available national-scale databases of known or potential emissions locations, including waste sites, AFFF-use sites, and industrial manufacturing plants. We will present case studies from two states (Michigan and New Hampshire), where ground-truthing of sources with local agency officials was used to refine emissions inventories. We discuss directions forward for better understanding atmospheric PFAS sources and deposition and key data gaps.

**4.03.P Airborne and Volatile Per- and Polyfluoroalkyl Substances: Measurements and Recent Developments**

**4.03.P-Tu077 Development and Demonstration of Volatile and Semi-Volatile Per- and Polyfluoroalkyl Substances (PFAS) GC-MS Methods on Select NIST Reference Materials**

***Alix E. Rodowa** and Jessica Lynn Reiner, National Institute of Standards and Technology (NIST)*

Per- and polyfluoroalkyl substances (PFAS) are emerging contaminants of concern, and have been observed in consumer products, consumer byproducts (e.g., dust, wastewater, and landfill leachate), and environmental compartments including humans and biota. As a result, routine monitoring methods, method development, and commercial analysis of these compounds have become more widely available. Current commercial methods typically target ionic, less volatile PFAS by liquid chromatography tandem mass spectrometry (LC-MS/MS). However, volatile and semi-volatile analytes are not commonly included despite the fact that volatile and ionic PFAS are known to co-occur as a result of manufacturing processes. Further, volatile PFAS are commonly excluded due to their poor sensitivity by LC-MS/MS and a lack of materials available to validate analytical methods (e.g., Standard Reference Materials (SRMs) and analytical standards). To fill this data gap, multiple instrumental methods for twelve volatile and semi-volatile PFAS including 4:2, 5:2s, 6:2, 7:2s, 8:2, and 10:2 fluorotelomer alcohols, 8:2 and 10:2 perfluoralkyl acrylates and perfluoroalkyl acetates, and methyl- and ethylperfluoroctanesulfonamide, have been developed for electrospray ionization gas-chromatography mass spectrometry (EI GC-MS). To demonstrate the efficacy of the methodology two Standard Reference Materials (SRMs) from NIST, SRM 2585 Organic Contaminants in House Dust and SRM 2781 Domestic Sludge were selected for quantitative evaluation of GC amenable PFAS. Each of these materials represents a different analytical compartment, and both materials have reported concentrations of ionic PFAS by LC-MS/MS on their Certificates of Analysis. These volatile PFAS measurements add value to the volatile PFAS knowledge-base and provide needed methodological, analytical validation materials.

**4.03.P-Tu078 Atmospheric deposition of per- and polyfluoroalkyls substances (PFAS) in Miami-Dade, South Florida**

***Maria Guerra de Navarro**, Natalia Soares Quinete and Yosmely Reyna, Florida International University*

Per- and polyfluoroalkyls Substances (PFAS) were first synthesized over 60 years ago and continue to be in use due to the exceptional stability of C-F bonds, being part of many consumer products and particularly of aqueous firefighting foams (AFFF). These compounds are water soluble and resistant to biodegradation and, therefore, have become extremely persistent in the environment. Their inability to be removed by traditional water treatments and the health risks associated with exposure to PFAS have brought the need to better understand its fate and transport in the environment. The major PFAS sources are related to human activities, after the compound has been released it enters the water cycle and follows different transport mechanisms. Atmospheric deposition is considered an important source and pathway for PFAS transportation, especially in areas far away from production sites, it could be comparable to or higher than point source inputs. The nucleation and wash-out of pollutants associated with wet deposition promote its spread towards long distances from the point source. In the state of Florida, major point sources of PFAS are facilities where the AFFF was heavily used in the past, such as military bases, firefighting training facilities, and airports, landfills, and wastewater discharges. This work seeks to assess the occurrence and composition of semi volatile and volatile PFAS present in wet deposition in the Miami-Dade area, South Florida. For this purpose, samples were taken in two locations in Miami-Dade County from October 2021 to September 2022 (N=25), encompassing the rainy and dry seasons. The methodology for sample preparation involves the preconcentration by solid phase extraction process (SPE) using a weak anion exchange (WAX) cartridge, followed by liquid chromatography tandem mass spectrometry analysis (LC-MS/MS), using isotopically labeled internal standards. 250 mL of rainwater were used for analysis and the methodology already established in the lab for the analysis of PFAS in surface and tap waters was further validated for rainwater samples, assessing method precision, accuracy, and matrix effects. The results show higher percentage (63-76%) composition of long chain PFAS (more than 8 carbons), and perfluoroalkyl carboxylic acids (PFCA) as the major components (47-94%), whereas Perfluoro-n-undecanoic acid (PFUdA) was the most frequent detected compound and its highest concentration was up to 14 ng. $L^{-1}$.

### 4.03.P-Tu079 Method development for detection of volatile PFAS by Thermo Desorption GC/MS

***Kevin Huncik**, Alix E. Rodowa, Jessica Lynn Reiner and John R. Kucklick, National Institute of Standards and Technology (NIST)*

Man-made contaminants in the environment frequently occur in highly complex mixtures of varying elemental composition. Thermal Desorption Gas Chromatography coupled with Mass Spectrometry (TD-GC/MS) has been shown to be a promising technique for the determination of volatile compounds; however, little has been done exploring per- and polyfluoroalkyl substances (PFAS) using this technique. Here we investigate, using TD-GC/MS, the enhanced detection and separation of volatile (and semi-volatile) PFAS. Further, we explore the feasibility of this technique for the determination of PFAS contained in textiles and environmental samples. Standards, textiles, and environmental samples were extracted using the Markes Microchamber and Thermal Desorber attached to an Agilent 8890 GC interfaced to the Agilent 5977B Mass Spectrometer. The GC column used was an Rtx-VMS (30m x 0.25mm x 1.4µm, Restek). The interfacing Markes Thermal Desorber with the GC/MS was successful in the preliminary analysis of volatile PFAS. All groups of compounds could be identified selectively, and sensitivity was different between the single compounds detected. The use of multiple types of sorbent tubes, for sorption of PFAS from the thermal desorber, were tested, allowing for the extraction and detection of several PFAS which cannot be identify by the more typical liquid chromatography tandem mass spectrometry methods. This presentation will discuss the method development efforts at NIST for the detection and quantification of volatile PFAS in textile and environmental samples using TD-GC/MS.

### 4.03.P-Tu080 Air Sampling and Analysis of Short-Chain Per- and Polyfluoroalkyl Substances

***Hua Wei**, Susan Wolf and Brian Mader, 3M Company*

The study of short-chain per- and polyfluoroalkyl substances (short-chain PFAS) in manufacturing and ambient environments requires proper sampling and analytical methods. In this study, an air sampling and analytical method was developed for the measurement of short-chain PFAS in air. We will present the results of the performance of this method for measuring C2-C7 PFAS in air. The method utilizes sorbent tubes containing XAD-2, XAD-4 or XAD-7. After sampling air through an adsorbent, the collected PFAS are extracted with solvent and the extracts analyzed using high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC/MS). The following parameters were evaluated (1) Performance of the air sampling tubes for short-term and long-term sampling applications (2) Performance of the different adsorbents for the different PFAS compounds. (3) The stability of the C2-C7 PFAS in the different organic solvents and on the various sorbents. Particularly interesting observations regarding the stability of trifluoroacetic acid (TFA) and perfluoropropanoic acid (PFPA) in methanol will be presented. In addition, challenges related to the HPLC/MS based analysis of ultra-short-chain compounds (C2-C3) will be discussed and analytical configurations to resolve these challenges presented.

### 4.03.P-Tu081 Per- and Polyfluoroalkyl Substances (PFAS) in Air in the Canadian Arctic and Great Lakes

*Fiona Wong[1], Chubashini Shunthirasingham[1], Wen-Long Li[1], Richard Park[1], Patrick Lee[1], Phil Fellin[2], Henrik Li[2], Alexander Vlasenko[2] and **Hayley Hung**[1], (1)Environment and Climate Change Canada, (2) AirZone One Ltd., Canada*

Per- and polyfluoroalkyl substances (PFAS) have been measured in air in the Canadian High Arctic Station of Alert (82° 30′ N 62° 20′ W) since 2006. The targeted PFAS for air samples included i) neutral PFAS: 6:2, 8:2, 10:2 fluorotelomer alcohols (FTOHs), 6:2, 8:2, 10:2 fluorotelomer acrylates (FTAs), methyl and ethyl perfluorooctane sulfonamides (Me- and EtFOSA), and methyl and ethyl perfluorooctane sulfonamidoethanols (Me- and EtFOSE) and ii) perfluoroalkyl acids (PFAAs), including C4-14, C16, C18 PFCAs and C4, C6, C8, C10 perfluoroalkyl sulfonic acids (PFSAs). Time trends of perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) both peaked in 2013 followed by steady declining trends; with halflives calculated from the peak in 2013 to 2017 as 4.8 years and 2.8 years, respectively. The declining trends probably reflect continued efforts to phase out these two compounds globally. Perfluorohexane sulfonic acid (PFHxS) appeared to decline from 2013 but this may be driven by a few high concentration measurements in 2013 and low measurements in 2017. Perfluorononanoic acid (PFNA) showed non-changing trends and perfluorodecanoic acid (PFDA) and perfluoroundecanoic acid (PFUnDA) showed increasing trends. Assessment of trends for other short- and long-chain PFAAs was hampered by their low detection frequencies and inconsistent blank values. Their trends need to be confirmed with further measurements in future years and the identities of some compounds need to be confirmed with high-resolution analysis.

XAD-based passive air samplers (XAD-PASs) were deployed at: i) 7 Arctic communities, namely Iqaluit, Nain, Northwest River, Kuujjuaq, Cambridge Bay, Inuvik and Fort Resolution; ii) 3 Great Lakes sites, namely Manitoulin Island, Georgian Bay Islands and Point Pelee; and a reference suburban site at Downsview close to Toronto. Neutral PFAS were analyzed in samples collected from 2016 to 2020. Only 6:2, 8:2 and 10:2 FTOHs were detectable in these samples. Generally speaking, concentrations of FTOHs in Arctic air were about a factor 2-3 lower than those found in air in the Great Lakes; with the highest concentrations found at the suburban site of Downsview.

**4.03.P-Tu082 Exploring the Drivers of Perfluorocarboxylic Acid (PFCA) Gas-Particle Partitioning Using a Model and Observational Constraints**

*Ye Tao, Trevor VandenBoer, RenXi Ye and **Cora Young**, York University, Canada*

The atmospheric fate of perfluorocarboxylic acids (PFCAs) has attracted much attention in the recent decades due to the role of the atmosphere in global transport of these hazardous chemicals. There is a gap in our understanding of gas-particle partitioning, limited by availability of reliable atmospheric measurements, partitioning properties, and models of gas-particle interactions. The gas/particle phase partitioning of C2-C14 PFCAs in the atmosphere are here modeled by taking account of both deprotonation and phase partitioning equilibria among air, aerosol liquid water, and particulate water-insoluble organic matter using a range of available PFCA partitioning properties. We systematically varied water and organic matter content to simulate the full range of atmospheric conditions. Except in severe organic matter pollution episodes, shorter-chain PFCAs are predicted to mainly partition between air and aqueous phase, while PFCAs with carbon chains longer than 12, organic matter is more likely to be the dominant particle phase sink. The model framework underestimated the particle fraction of C2-C8 PFCAs compared with several ambient observations, with larger discrepancies observed for longer-chain PFCAs. One to three orders of magnitudes higher particle/gas equilibrium ratios are required to fulfill the agreement. The discrepancy could result from externally mixed dust components, non-ideality of aerosol liquid water, and missed interactions between organic matter and charged PFCA molecules. Reliable measurements of ambient PFCAs with high time resolution and the measurement of uptake parameters by particle-relevant components will be beneficial to more reliable environmental fate modeling of ambient PFCAs.

**4.04.P Analysis of Pharmaceuticals, Pesticides, and Other Chemicals in Environmental Matrices**

**4.04.P-Mo097 Changes in Fine Particulate Matter Composition During Preparation for Toxicity Testing**

*Emma Weeks[1], Lisandra Trine[1], Staci Simonich[1] and **Courtney Roper**[2], (1) Oregon State University, (2) University of Mississippi*

Exposure to the complex mixtures present in fine particulate matter ($PM_{2.5}$) result in variable adverse human health effects, dependent in part due to $PM_{2.5}$ composition. Understanding the causal components and underlying mechanisms of the known health associations is a critical step in protecting human health. Toxicology studies are necessary to answer these questions and are frequently conducted using filter collected $PM_{2.5}$. However, the methods of $PM_{2.5}$ filter extraction have significant impacts on both the chemical composition and subsequent toxicity, highlighting the importance of standardized, streamlined methods. Our goal is to optimize a previously used filter extraction method for $PM_{2.5}$ toxicology research by quantifying chemical constituent losses throughout the steps of sample processing, including filter extraction and preparation for toxicology studies, for $PM_{2.5}$ collected from different sources (n=3). Four different points during the sample processing were analyzed for polycyclic aromatic hydrocarbons (n=118) and elements (n=75) via gas chromatography-mass spectrometry and inductively coupled plasma optimal emission spectrometry, respectively; the points during extraction were following: sonication, concentration, drying, and re-suspension in a media amenable to toxicity testing (DMSO). Concentrations of chemical constituents at the various points in the sample preparation process were compared to constituent concentrations using standard extraction methods for characterization of ambient $PM_{2.5}$. Stark differences were observed between the preparation steps in concentrations of PAH classes, with differences between steps being over 100-fold in some cases. The compositional profiles varied between steps and sources of $PM_{2.5}$. Elemental data and differences based on the sources of $PM_{2.5}$ are currently being analyzed to ultimately optimize sample preparation of filter collected $PM_{2.5}$ for toxicity studies. This work will contribute to the growing evidence that filter extraction methods for $PM_{2.5}$ must be standardized due to potential compound losses during different preparation steps. Ultimately, this work will support accurate inter-laboratory comparisons of $PM_{2.5}$ toxicity that will lead to improved air regulations to protect human health.

**4.04.P-Mo098 Fate and Transport of Nanopesticides in Field Scale Agricultural Applications**

***William Dallas Rud***[1]*, Tiffany Messer*[1]*, Manuel David Montaño*[2] *and Daniel Miller*[3]*, (1) University of Kentucky, (2) Western Washington University, (3) U.S. Department of Agriculture*

Pesticide applications are a necessary part to increasing agricultural production for the growing world population, but bulk pesticide application has resulted in environmental contamination, human toxicity, and endangerment to non-target species (i.e, honeybees, monarch butterflies). Nanoengineered particles (ENP) are a potential solution with increased efficiency, longer duration, and enhanced stability of pesticides. However, the degradants and accumulation points in agroecosystems is still unknown. Therefore, the goal of this project was to evaluate the fate, transport, and persistence of two nanopesticides (copper(II) hydroxide and nano-imidacloprid) and standard imidacloprid within soils and runoff water of agricultural systems in Central Kentucky. It is hypothesized the nanopesticides would remain in the soil with the potential for toxic byproducts produced from interaction with the soil and pesticides. The study will be accomplished with a field scale application of the pesticides at recommended usage using 2.4 m by 6.1 m plots enclosed with metal boundaries. Surface water and soil column samples will be collected throughout the growing season and following rainfall event. Samples will be analyzed for nutrient, pesticide, and pesticide byproducts along with physiochemical soil/water characteristics (i.e., pH, specific conductivity, temperature). Findings from this project will provide guidance for potential interactions of the nanopesticides within agricultural and downstream ecosystems and potential contaminant concerns. Further, findings will provide guidance for nanopesticide regulation and application standards to prevent environmental issues.

**4.04.P-Mo101 Direct and Indirect Effects of Chronic Venlafaxine Exposure on a Freshwater Ecosystem**

***Heather Jovanovic***[1]*, Jonathan Karl Challis*[2]*, Vince Palace*[3]*, Mark L. Hanson*[1] *and Pepe Rodriguez-Gil*[4]*, (1) University of Manitoba, Canada, (2) University of Saskatchewan, Canada, (3) IISD Experimental Lakes Area (IISD-ELA), Canada, (4) International Institute for Sustainable Development, Canada*

Venlafaxine is an antidepressant drug that is present in freshwater ecosystems worldwide with detections of >2.0 µg/L. Its effects have been well studied in laboratory settings and it is known to alter behavioural responses in fish. However, its potential effects have not been investigated in a whole ecosystem context where direct and indirect effects of chronic exposure could pose a risk to long-term ecosystem health. To assess the potential risk of chronic venlafaxine exposure, limnocorrals (n=10, 2-m diameter, 1.5-m deep) were deployed in a lake at the IISD-Experimental Lakes Area, spiked weekly with venlafaxine for 10 weeks and monitored for various biotic and abiotic responses. Limnocorrals contained a native assortment of plankton and invertebrates and were each stocked with finescale dace (*Chrosomus neogaeus*), a native small-bodied littoral fish. Here we show the behavioural responses of fish and indirect responses of native plankton and invertebrate communities to chronic venlafaxine exposure. For most endpoints, minimal differences were observed at environmentally relevant concentrations. The concentrations tested were not directly lethal to aquatic biota. Indications of behavioural changes in fish suggest reduced predation pressure may have caused indirect effects on invertebrate community structure. This study integrates the whole ecosystem response to a commonly detected freshwater contaminant and found that venlafaxine at environmentally relevant concentrations does not appear to pose a significant ecological risk to aquatic ecosystems.

**4.04.P-Mo102 Evaluating the reusability of Tenax in Estimates of Bioaccessibility through Single-point Extractions**

***Sam Nutile*** *and Yucheng Shao, Penn State Behrend*

Single-point Tenax extractions are a viable means of estimating bioaccessibility of hydrophobic organic contaminants in sediment, soil, and intestinal fluids. One advantage of this extraction technique is that after thorough cleaning and drying, Tenax beads can be reused in subsequent extractions with the assumption that no changes in bioaccessibility estimates will occur. This assumption of reusability, however, has not been tested in the context of single-point extractions. Therefore, the objective of the current study was to evaluate the reusability of Tenax beads by comparing bioaccessible polychlorinated biphenyl (PCB) concentrations measured by differently aged Tenax. New Tenax beads (60-80 mesh) were aged through 24 h single-point Tenax extractions of clean sand 0, 1, 5, 10, 15, 20, and 25 times. The aged Tenax was then used to extract 27 PCB congeners from laboratory spiked sediment and the bioaccessible PCB concentrations were compared. Despite differently aging the Tenax beads, there were no significant differences in bioaccessible PCB concentrations for most of the PCB congeners. Scanning electron microscope imaging revealed no significant changes in the size of the Tenax beads after aging, suggesting no significant changes in the Tenax phase volume resulting in consistent estimates of bioaccessibility through repeated use. Considering the strong correlations between single-point Tenax extractable concentrations and bioaccumulation by various organisms exposed to contaminated environmental matrices, providing data to detail the reusability of Tenax in repeated extractions further demonstrates the applicability of this extraction technique in risk assessment.

**4.04.P-Mo103 An Environmentally-Friendly Contraceptive Pill? The Estetrol-Based Pill as a Good Candidate**

***Sebastien Baekelandt***[1]*, Céline Gerard*[2]*, Céline Delierneux*[2]*, Nathalie Leroux*[1]*, Valérie Cornet*[1] *and Patrick Kestemont*[1]*, (1) University of Namur, Belgium, (2) Estetra SRL, Belgium*

Estetrol (E4) was recently approved in a combined oral contraceptive containing E4 15 mg (as monohydrate) and drospirenone (DRSP) 3 mg. This natural estrogen, produced by the human fetal liver during pregnancy, is also under development for use in hormone replacement therapy. It has a low potential for bioaccumulation in aquatic species, high mobility in soil and low persistence. E4 may also represent a lower risk to the aquatic environment than synthetic estrogens such as ethinylestradiol (EE), which are known to be potent endocrine disruptors in aquatic organisms. However, the characterization of the endocrine disruptive potential of E4 has not yet been assessed. The present study determined its potential chronic lethal and sublethal effects in an early-life stage toxicity test following the OECD test guideline 210. Zebrafish (*Danio rerio*) were exposed, from hatch to 1 month-post fertilization, to E4 and EE, with or without the progestin DRSP under flow-through conditions. It included 1 to 10,000-fold environmentally relevant concentrations of each molecule (11, 0.3 and 10 ng/L for E4, EE and DRSP respectively). Endpoints included hatching success, survival, growth and larval behavior. Furthermore, samples were collected for label-free quantitative proteomics and specific gene expressions. EE at 30 ng/L impacted growth, survival and larval behavior. Almost 300 proteins were also differentially expressed in fish exposed to EE, with several biological processes highlighted such as the cellular response to estrogen stimulus. In contrast, no differences in growth, survival, hatching success or behavior were observed with E4 at concentrations ranging from 11 ng/L to 11 μg/L. Concerning proteomics, approximately 100 proteins were detected as differentially expressed in response to E4, but no significant enrichment was obtained. In parallel to fish reproduction assays, the results obtained so far suggest that the combination of E4/DRSP acts as an environmentally-friendly alternative to synthetic estrogens.

**4.04.P-Mo104 The Use of Silicone Bands as a Passive Sampler for the Detection of Organic Contaminants in Coastal Waters**

***Brian Shaddrix***[1]*, Ed Wirth*[2]*, Marie E. DeLorenzo*[2]*, Andrew Wunderley*[3] *and Cheryl Carmack*[3]*, (1) CSS Inc./NOAA, (2) National Oceanic and Atmospheric Administration (NOAA), (3) Charleston Waterkeeper*

Passive samplers can be a useful tool to determine the presence of organic contaminants in aquatic environments. Compared to traditional passive samplers however, silicone bands are a relatively new technology that researchers are turning to because of their affordability and ease of use when conducting field monitoring. Although literature exists on research conducted in fresh water aquatic environments, there is little information regarding the use of silicone bands in the marine environment. In this study, we examine yearly seasonal patterns of organic contaminants targeting polycyclic aromatic hydrocarbons (PAH's), pyrethroids, and fipronil at 20 estuarine sites in and around Charleston, SC. The silicone band deployment sites are paired with sites currently monitored by Charleston Waterkeeper (a non-profit community interest group) for coliform bacteria and general water quality with the objective to determine potential differences among sites, between seasons, and year to year comparisons. The design includes deployment of bands for 28 days each season for 2 years. Preliminary results, including 2021 and part of 2022 field deployments, suggest that silicone bands can be used to detect measurable amounts of organic contaminants, and that when making comparisons between years, seasons, and field sites as well as between compound classes, noticeable differences are present. PAH 50 levels and Pyrethroid levels are more prevalent in the spring for example. This passive sampler technology will be further advanced by establishing uptake rate constants for classes of contaminants. The laboratory-derived rate constants will then be applied to estimate water concentrations from the chemicals adsorbed on the field-deployed silicone bands.

**4.04.P-Mo105 Development and validation of a modified QuEChERS method for extracting polychlorinated biphenyls and organochlorine pesticides from marine mammal blubber**

***Adam Pedersen***[1]*, Rune Dietz*[2]*, Christian Sonne*[2]*, Lan Liu*[1]*, Aqqalu Rosing-Asvid*[3] *and Melissa McKinney*[1]*, (1) McGill University, Canada, (2) Aarhus University, Denmark, (3) Greenland Institute of Natural Resources*

The monitoring of legacy persistent organic pollutants (POPs) in the blubber/adipose of key sentinel marine mammal species has been successfully conducted using established techniques for several decades. Although these routine methods for polychlorinated biphenyl (PCB) and organochlorine (OC) pesticide determination provide accurate and reproducible results, they possess some notable drawbacks in terms of cost, time and labor intensiveness, and a need for large-volumes of toxic solvents. To address these issues, we developed, validated, and applied a modified QuEChERS (quick, easy, cheap, effective, rugged, and safe) extraction method for the analysis of an extensive suite of PCB and OC contaminants in marine mammal blubber. We tested multiple solid-phase extraction (SPE) and clean-up steps to determine the approach that provided the cleanest extracts along with consistent and acceptable analyte recovery, accuracy and precision using GC-MS for quantification. QuEChERS liquid-liquid extractions followed by two effective matrix removal (EMR)-lipid, one primary-secondary amine (PSA), and one silica gel cartridge clean-up showed the highest matrix removal along with acceptable recoveries of spiked internal standards (69-94%) from recovery experiments. Solvent usage was reduced by ~393%, equating to ~$34 CAD less in solvent costs per sample relative to current-use methods. The optimized method was then validated using standard reference material (SRM) NIST 1945 organics in whale blubber and comparisons to current-use methods were conducted using southeast Greenland killer whale samples (n=13), with previously reported PCB and OC pesticide concentrations. Recovery experiments on SRM 1945 (n=5) showed high recovery (>70%) for 80% and 77% of PCBs and OC pesticides, respectively, and high precision (<20% relative standard deviation) for 76% and 77% of PCBs and OC pesticides, respectively. Bland-Altman plot analysis indicated good agreement between QuEChERS and the current-use methods for ΣPCBs and ΣDDTs with no significant constant or proportional bias. However, significant constant bias was present for

ΣCHLs and significant proportional bias was present for ΣHCHs. These results demonstrate that QuEChERS-based analyses for PCB and OC pesticides in blubber, and likely other high-lipid samples, represents an effective, lower cost alternative to current-use extraction methods.

**4.04.P-Mo106 Some like it hot - Warmer Water Temperatures Improve Sea Lamprey's (Petromyzon marinus) Survival to Lampricide Exposures**

***Dejana Mitrovic***[1], *Hugo Flávio*[2], *Leslie Bragg*[1], *Mark R. Servos*[1] *and Michael Wilkie*[2], *(1) University of Waterloo, Canada, (2) Wilfrid Laurier University, Canada*

Since the 1960s, there has been a focused effort by the Great Lakes Fisheries Commission to control populations of invasive sea lamprey (*Petromyzon marinus*) in the Laurentian Great Lakes to protect commercial, recreational and culturally significant fisheries from lamprey predation. Applications of the lampricide, TFM (3-trifluoromethyl-4'-nitrophenol) to larval sea lamprey infested streams and rivers have been highly successful in reducing population numbers due to the sea lamprey's greater sensitivity to this pesticide. Most fishes exposed to TFM can more effectively detoxify it using Phase II detoxification, in which TFM is biotransformed into a much less toxic compound that is easier to excrete. Sea lamprey are highly sensitive to TFM exposures because they have limited detoxification ability, resulting in the rapid accumulation of TFM in the tissues and death. Environmental factors such as increases in water pH and higher alkalinity can significantly reduce TFM effectiveness by decreasing TFM bioavailability. However, we recently observed that TFM tolerance also increases as water temperature increases. We propose that as water temperature increases, TFM toxicity is reduced due to an increased ability of sea lamprey to detoxify and excrete biotransformed chemical. To test this hypothesis, toxicity tests were performed on larval sea lamprey after long-term acclimation (3 weeks) to a wide range of water temperatures (7 to 28°C). The results indicate that the 12-h $LC_{50}$ of TFM to larval sea lamprey steadily increases with temperature, with characteristic rightward shifts of TFM dose-response curves. Measurements of tissue, liver and muscle, TFM concentration using LC-MS/MS support the hypothesis that sea lamprey's ability to survive TFM treatments at warmer temperatures is due to an enhanced capacity to detoxify TFM as water temperatures increase. Our findings suggest that in an ever-warming climate, higher sea lamprey tolerance to TFM will necessitate the use of higher concentrations of this lampricide for successful sea lamprey control, but this could result in a greater risk to non-target organisms, which will have to deal with higher environmental concentrations of TFM that might exceed their capacity to detoxify the compound.

**4.04.P-Mo107 D. Pulgarin-Zapata*, J.S. Bumagat, L.M. Bragg, P. Marjan, K. R. Munkittrick, M.R. Servos, V. Arnold, N. Ruecker, and M. J. Arlos.**

***Daniela Pulgarin Zapata***[1], *Jorge Seth Bumagat*[1], *Leslie Bragg*[2], *Patricija Marjan*[3], *Kelly Munkittrick*[3], *Mark R. Servos*[2], *Victoria Arnold*[4], *Norma Ruecker*[4] *and Maricor Jane Arlos*[5], *(1) University of Alberta, Canada, (2) University of Waterloo, Canada, (3) University of Calgary, Canada, (4) City of Calgary, Canada, (5) Department of Civil and Environmental Engineering, Canada*

The physico-chemical properties of the vast majority of micropollutants render them susceptible to various mass transfer and transformation processes such as biodegradation, photodegradation, and sorption. Provided that appropriate environmental conditions exist (e.g., biomass, sunlight, and particulate matter), these mechanisms can be highly effective in reducing the concentrations of micropollutants in the aquatic environment. Whole-system studies that assess fate and transport are important to develop an understanding of micropollutant behaviour. In contrast with lab-based studies, these systems incorporate the natural conditions that are associated with contaminant attenuation. The Advancing Canadian Wastewater Assets facility in Calgary (Canada) is equipped with 12 naturalized artificial streams (320 m long) integrated with a fully operating municipal wastewater treatment plant. The streams have hydraulic and ecological parameters that mimic natural local systems. This study investigates the partitioning of a diverse group of micropollutants, including pharmaceutical and personal care products in replicated artificial streams and five environmental compartments: water, sediments, invertebrates, biofilm, and fish. In addition, it aims to evaluate the impact of different types of

wastewater treatment (e.g., ultrafiltration, ozonation, reverse osmosis) on the occurrence and partitioning of these compounds in the environment. To address the study questions, three methods were used to extract the environmental samples: solid phase extraction (SPE) for stream and wastewater samples, accelerated solvent extraction (ASE) for sediments, and QuEChERS for biofilm, invertebrate, and fish tissues. The extracts were analyzed via liquid chromatography, triple quadrupole mass spectrometry (LC/MS-MS). Preliminary results of this study show that many compounds including sulfamethazine, carbamazepine, ibuprofen, venlafaxine, sulfamethoxazole, oxybenzone, triclosan, gemfibrozil, naproxen, diclofenac, trimethoprim and atorvastatin are found in the water compartment. Additional analyses are currently underway to determine whether bioaccumulation factors in biofilms, fish, and invertebrates can be obtained.

**4.04.P-Mo108 Development of an Enantiospecific Analytical Method for the Detection and Quantification of Chiral Pharmaceuticals in Wastewater-impacted Systems**

***Sondus Jamal****, Leslie Bragg, Wayne Parker and Mark R. Servos, University of Waterloo, Canada*

Pharmaceuticals and personal care products (PPCPs) are increasingly being detected in treated wastewaters and in receiving waters at trace levels (i.e. µg/L to ng/L). Many PPCPs are chiral and are commonly administered as racemic mixtures (e.g. venlafaxine). In environmental matrices, they are typically quantified by liquid chromatography and triple quadrupole mass spectrometry (LC-MS/MS) using an achiral column. However, the use of an achiral method does not facilitate the distinction between enantiomers of chiral PPCPs. The development of reliable analytical methods for the identification and quantification of each enantiomer is crucial for studies of the enantiospecific behaviour of chiral PPCPs in the environment. An enantiospecific LC-MS/MS method has been developed and validated for the detection and quantification of venlafaxine (VEN), O-desmethylvenlafaxine (DesVEN), fluoxetine (FLX), norfluoxetine (NFLX) and atenolol (ATEN) in their enantiomeric forms (R and S configurations) in raw and treated wastewater and river water using the Grand River, Ontario, Canada as a platform. River and wastewater samples were filtered and, with the exception of atenolol samples, adjusted to a pH of 2 prior to solid phase extraction (SPE) to enhance recovery. The MS/MS was optimized using racemic standards to confirm compound detection, and to optimize other parameters (e.g. collision energy of the parent ion, and energy of the fragmented ion). LC parameters such as mobile phase composition/concentration, pH, additives, flowrate, and temperature were optimized to obtain satisfactory sensitivity, reduced matrix effects and high enantiomeric resolution. The optimized and validated method utilizes the Chiral-V column (2.7µm, 4.6x100mm) at 25°C, and run with an isocratic mobile phase (4mM ammonium acetate in methanol with 0.005% formic acid) at a flow of 0.5mL/min, injection volume of 10µL, run duration of 20 minutes, and using multiple reaction monitoring (MRM) with 200 milliseconds dwell time and widest resolution. The instrument was calibrated using racemic standards at concentrations ranging from 0.5 to 3000 µg/L, and the enantiomeric configuration of the eluted peaks was confirmed using R and S only standards. This method enables fast, reliable, sensitive, and unbiased quantification of the enantiomers of VEN, DesVEN, FLX, NFLX and ATEN, which is crucial when trying to investigate their fate and environmental risk.

**4.04.P-Mo111 Strategies and Challenges for Conducting Fish Nature of the Residue Studies**

***Emma Danby*** *and Lindsay Knight, Labcorp Early Development Laboratories Limited, United Kingdom*

With the increasing popularity of fish-based diets, a large proportion of fish we eat come from fish farms. The diet used in these farms often comprises a substantial portion of plant commodities leading to a potential risk of exposure to pesticide residues. The European pesticide regulation requires fish metabolism and fish feeding studies where residues in fish feed exceed 0.1 mg $kg^{-1}$ of the total diet (dry weight basis), hence enabling the setting of appropriate maximum residue levels in fish commodities.

At Labcorp, we are using our extensive experience of conducting fish bioaccumulation studies (both aqueous and dietary exposure, OECD 305) and livestock nature of the residue (OECD 503) to establish strategies for overcoming the potential challenges of the fish metabolism study (SANTE/10254/2021 23 February 2021 Nature of pesticides residues in fish).

Here we present the preliminary results of our first in-house fish residue studies.

**4.04.P-Mo112 Extraction of Nitrite and Nitrate from Swine Tissues following Oral Administration of Sodium Nitrite**

***Gerald Sanders**[1], Emily Ruell[2] and Amos Kwabena Dwamena[1], (1) Smithers, (2) U.S. Department of Agriculture*

Sodium nitrite ($NaNO_2$), like other common salts, is regularly used as a meat preservative to inhibit microbial growth during processing and curing.  However, when consumed in high doses over a short period of time, sodium nitrite can cause severe methemoglobinemia in mammals, where the ability of blood to transfer oxygen to tissues is markedly reduced, and has been shown to be toxic to feral swine (*Sus scrofa*).  Feral swine are of particular concern in agriculture as they are an invasive species which contribute to millions of dollars in losses to agricultural crops each year. A sodium nitrite-based toxicant in development for the control of feral swine has been designated as a food use pesticide by the EPA.  The potential use of sodium nitrite within a bait for destructive feral swine could pose a concern in the unlikely event that a feral swine is hunted and used as food shortly after it consumed the bait.  A study for evaluating sodium nitrite residues in edible tissues of domestic pigs as a surrogate for feral swine has been conducted in conjunction with USDA APHIS to help in the assessment of human health impacts.  In addition to an overview of study design, particular focus will be spent on the extraction and HPLC-UV analysis of sodium nitrite and its oxidative product, sodium nitrate, from small intestines, liver, muscles, and adipose tissues, along with discussion on the challenges encountered.  In addition, the magnitude of nitrite/nitrate residue levels in the different tissue types will be summarized.

**4.04.P-Mo113 Identification of Pesticides in Vernal Pools Using Suspect Screening Methods to Improve Exposure Assessment of Listed Species**

***Amanda Brennan** and Leah Oliver, U.S. Environmental Protection Agency*

Vernal pools are ephemeral wetlands which provide critical habitat for uniquely adapted organisms that can withstand variable periods of hydration and drying.  The fairy shrimp, *Branchinecta lynchi* (*B. lynchi*), is one such organism that is listed as threatened under the Endangered Species Act.  In California, vernal pools are often located near agricultural fields and can be impacted by pesticides from application and runoff.  Fairy shrimp can be exposed to pesticides during hatching and throughout their lifespan.  In efforts to develop exposure and effects assessments for *B. lynchi* and demonstrate approaches to improve the risk assessment process for listed species, paired water and sediment samples were collected from vernal pools in central California.  An analyte list of 13 selected pyrethroids and organophosphate pesticides (OP) for targeted analysis by gas chromatography tandem mass spectrometry (GC/MS/MS) was generated based on pesticide application and crop data from surrounding agricultural areas.  Chemical analysis of the water samples, however, resulted in non-detectable concentrations of pesticides by this method with detection limits of approximately 1 ng/mL.  Therefore, a suspect screening method was adapted to screen for more than 220 pesticides to potentially identify other unknown pesticides in paired sediment samples and subsequent water analyses.  The suspect screening method consists of pesticides from GC/MS/MS amendable classes including pyrethroids, OPs, organochlorines, carbamates, organonitrogens, and herbicide methyl esters using full scan and selected reaction monitoring (SRM) in electron ionization (EI) mode.  Blind method validation was conducted on spiked standards prior to analysis of samples. Validation samples were spiked at low and high concentrations of approximately 1 to 5 and 20 to 30 ng/mL, respectively, depending on pesticide molecular weight.  For the low and high concentration validation samples, 80% and 100% of the pesticides were identified, respectively, with fewer identifications in the low concentration samples likely being from detection limits. All internal standards were identified in validation samples.   Analysis of paired sediment samples and subsequent water analyses using the targeted and expanded suspect screening method can provide a more detailed picture of pesticide exposure to *B. lynchi* in vernal pools for risk assessment.  *Abstract does not reflect Agency policy.*

**4.04.P-Mo114 Chlorinated Paraffins: Environmental Occurrence of Chlorinated Paraffins in Major Components of the Environment – Water, Wastewater, Solids, and Tissue.**

***Million Woudneh**, Bharat Chandramouli and Coreen Hamilton, SGS AXYS Analytical Services Ltd., Canada*

Chlorinated paraffin (CPs) are high-volume production chemicals with widespread applications as lubricants, flame retardants, and plasticizers. Some researchers have identified CPs as the most difficult halogenated compounds to analyze and quantify. In this project, we have developed a simplified method that employed ultra-high performance liquid chromatography and tandem mass spectrometry to quantify short-chain (C10 – C13, SCCPs), medium-chain (C14–C17, MCCPs), and long-chain (C18-20, LCCPs) chlorinated paraffins. Replicate spike/recovery experiments revealed the method to have good accuracy (88–127%) and precision (<12% RSD) in all matrices. Chlorinated paraffin concentrations published in the open literature for known standard reference materials was used as external validation of the method and generated good agreements. Spiked experiments were also conducted to study potential interferences from 209 PCB congeners, 29 chlorinated pesticides, and technical toxaphene mix. The developed method was used for characterization of CPs in diverse environmental matrices such as surface water runoff, sediment samples, and solid and liquid streams of wastewater treatment plants (influent, effluent and biosolids) and tissue. The results generated demonstrated the applicability of the developed method for supporting diverse environmental monitoring.

**4.04.V Analysis of Pharmaceuticals, Pesticides, and Other Chemicals in Environmental Matrices**

**4.04.V-01 Passive sampling strategy for monitoring very persistent and very mobile substances**

***Yoonah Jeong** and Dong-Chul Shin, Korea Institute of Civil Engineering and Building Technology (KICT), Korea, Republic of (South)*

This study focuses on very persistent and very mobile (vPvM) substances in the environment to ensure safe and clean water quality. Once released, vPvM substances tend to pass through wastewater treatment plants into the environment due to the limited removal rate. Since they are not easily sorbed to organic matter, these substances remain in various environmental matrices for a long time. In a previous study, fifteen vPvM substances were prioritized based on various databases and the guideline of the European Commission. The boundary of vPvM substances is based on the Chemicals Strategy for Sustainability Towards a Toxic Free Environment of the European Commission. In this study, a passive sampling strategy is devised to reliably monitor vPvM substances in the water environment. Considering that these substances exist in the ng/L concentration range, sorption-based passive sampling devices such as polar organic chemical integrative samplers (POCIS) are included. Selection of passive samplers, sampling site, sampling period and analysis plan are organized targeting vPvM substances.

**4.04.V-02 The Advantages of Using Passive Sampling in Monitoring Veterinary Pharmaceuticals**

***Nikola Rakonjac**[1], Erwin Roex[2] and Henry Beeltje[3], (1) Wageningen University & Research, Netherlands, (2) National Institute for Public Health and the Environment (RIVM), Netherlands, (3) Netherlands Organisation for Applied Scientific Research (TNO), Netherlands*

Veterinary Pharmaceuticals are used world-wide to cure or prevent illness of animals, and their residues are frequently detected in the surface water. Typically, prior to analyses these compounds are collected with grab samples and consequently the measured concentrations refer to a moment of sample collection. To improve estimates of chronic exposure to these compounds and enhance the sensitivity of our sampling method, we performed a water quality sampling campaign with passive samplers. This type of measurements has the advantage of sampling over a longer time period and thus provides time-weighted average concentrations. Besides, by sampling over a longer period, the amount of water which is sampled is larger than for grab sampling, thus lowering the limit of detection. Our investigation focused on surface water in a typical agricultural region in the Netherlands, and we performed measurements at eight locations. As a passive sampler, we chose the Speedisk® $H_2O$-Philic DVB, as this type of sampler is more suitable for sampling the more polar compounds, like most of the Veterinary Pharmaceuticals. Totally, we targeted 46 compounds, among which 25 antibiotics, three hormones, nine antiparasitics, and nine disinfectants. Sampling strategy is based on having two pair of samplers at each location, where one pair is deployed for three months, and a second pair is changed every four weeks. This approach allows for determining the influence of manure applications patterns and various hydrological aspects (e.g. rainfall/runoff events). At all eight investigated locations the presence is confirmed for: the Antibiotics Flumequine, Sulfadiazine, Sulfamethoxazole, and Tilmicosine; the antiparasitic Flubendazole; the hormones Estrone, and Progesterone; and the disinfectants Benzyldimethyldodecylammonium chloride, and Didecyldimethylammonium chloride. Other compounds are either not detected (18) or are present occasionally at some locations (19). Sampler locations were recalculated to aqueous concentrations, and based on these concentrations, none of the individual detected veterinary pharmaceuticals exceeds the PNEC. However, a summed effect of the total mixture on certain locations cannot be excluded. Considering that some targeted compounds (i.e. disinfectants) are quantified for the first time in the Netherlands, our approach and results may be used as a first step in developing a targeted monitoring program for veterinary pharmaceuticals.

**4.04.V-03 Impacts of wastewater effl uents and seasonal trends of emerging contaminant in water and sediments of two cold-region rivers**

***Ana Cardenas Perez,*** *University of Saskatchewan, Canada*

Emerging contaminants such as pharmaceutical drugs have been detected in waters across the globe. Most of the pharmaceuticals are found at trace concentrations, but the continuous use and potential accumulation of some of these compounds can lead to potential effects in aquatic organisms, due to their continuous release, high biological transfer potential, and possible bioaccumulation in aquatic organisms. Wastewater treatment plants are not designed to remove pharmaceuticals, so these compounds are virtually unattained before the treated water is discharged. Consequently, WWTPs' management has a key role in the removal and monitoring of pharmaceutical products with challenges related to the polar nature of these chemicals that facilitate their presence in different environmental compartments. Pharmaceutical concentrations can be affected by temporal variations, the flow velocity of water receptors, the sorption capacity of sediments, and other abiotic dynamics in aquatic ecosystems. Currently, most experimental approaches have not considered these dynamics to evaluate the chemical activity, bioavailability, and toxicity of pharmaceuticals. The principal aim of this research is to enhance our understanding of the environmental risks associated with pharmaceuticals as one group of emerging contaminants. To this end, the presence of a suite of representative pharmaceuticals was measured upstream and downstream of two WWTPs located in the South Saskatchewan River basin and Wascana Creek, Saskatchewan, Canada, during three sampling campaigns (spring, summer, and fall of 2021), through both conventional and passive water sampling. According to the chemical analysis conducted, Amitriptyline with a concentration of 3353.8 ng/L was the most abundant compound in comparison to the other pharmaceuticals evaluated in the water and sediment samples at the four sampling sites and across the three seasons. The data collected from this monitoring campaign indicates widespread contamination with psychoactive pharmaceuticals, which can have marked impacts in exposed organisms.

**4.04.V-04 Removal of Pharmaceuticals from Source Separated Urine Using Locally Manufactured Biochars.**

***Pius Kinoti Kairigo***[1]*, Josephine Ouma*[2]*, Elijah Ngumba*[2]*, Austine Owuor*[3] *and Tuula Tuhkanen*[1]*, (1) University of Jyvaskyla, Finland, (2) Jomo Kenyatta University of Agriculture and Technology (JKUAT), Kenya, (3) Technical University, Kenya*

Human urine is a point source of pharmaceutical residues because Majority of active pharmaceutical ingredients of concern are excreted through urine in the original form or as active metabolites.

In conventional sanitation systems, urine is flashed and infinitesimally diluted with water causing the present pharmaceutical residues to occur in nanogram to below detectable levels in the wastewater streams. The conventional wastewater treatment systems are inefficient at treatment of micropollutants at these very low levels, therefore they are released to the receiving water bodies through the effluent. The sub-inhibitory concentrations of antimicrobials in the environment are a cause of concern because of the risk of development and propagation of antimicrobial resistance among environmental microorganisms.

Separation of human waste at source is gaining popularity across the globe, especially in the informal settlements, mainly as a measure to provide alternative sanitation to unsewered populations, or to supplement the already existing sanitation systems. Complete value addition streams exist based on separated human waste. However, prior treatment before use , especially for urine is necessary as a risk control measure. This ensures a micropollutant free product is generated that enters the environment.

In this study, biochars from selected biomass (coffee husk, Pineapple waste, dry fecal matter) were used to adsorb selected antibiotics (sulfamethoxazole, trimethoprim, norfloxacin) and antiretroviral drugs (Lamivudine, nevirapine, zidovudine) in urine. A commercial Wood based activated carbon was used for comparison. LC-ESI-MS/MS was used for analysis.

In our preliminary batch adsorption studies, commercial activated biochar indicated percentage removal >90 for all the pharmaceutical compounds. Coffee husk biochar had the best performance for the locally manufactured biochars with removal percentages ranging from 10-40. Pineapple waste, and dry fecal matter char had removal efficiencies below 10 percent. A process to audit the low removal efficiencies for the locally manufactured biochars is ongoing. The preparation protocols, particle size, carbonization temperatures and possible surface modification will be explored with an aim of improving the adsorption capacity and removal efficiency. The end goal is to prepare a biochar-based filter that is cheap, efficient and robust for the treatment of source separated urine at the community level.

**4.04.V-05 Organochlorine Pesticide Residues in Drinking Groundwater from Areas Around Agricultural Farms in Southern Nigeria: Occurrence, Levels, and Human Health Risk**

***Isioma Tongo*** *and Valentina Ehighaokhuo, University of Benin, Nigeria*

Drinking groundwater samples from agriculturally dominated areas in Edo State, Southern Nigeria were assessed for pesticide residues to evaluate pesticide occurrence, levels, and human health risks. 36 samples of borehole water were collected from August 2020 to January 2021 and analysed for pesticides using high-performance liquid chromatography (HPLC model CECIL 1010). Human health risk assessment was evaluated using human health risk assessment models. Total pesticide concentrations ranged from 0 – 0.268 µg/L in Station 1, 0 – 0.880 µg/L in Station 2 and 0 – 0.218 µg/L in Station 3. Station 1 (Weppa Farm, Aganebode) was the most contaminated site with mean values of 0.079 ± 0.11 µg/L accounting for 49.5 % of the total pesticide residues in the stations. Endrin was the most frequently observed pesticide with mean concentrations of 0.014 ± 0.018 µg/L. Monthly variation showed higher levels of total pesticide residues in September with mean concentrations ranging from 0 to 0.268 µg/L. The contamination levels were generally low, however, pesticide levels in some of the water samples exceeded the European Drinking Water Standard of 0.1 µg/L. Estimated health risk for the overall non-carcinogenic and carcinogenic health effects showed that the contamination of the groundwater samples had low risks. Since OCPs can accumulate in the groundwater over time due to their persistence, regular monitoring of groundwater around agricultural farms is therefore imperative to prevent risks to human health.

**4.04A Analysis of Pharmaceuticals, Pesticides, and Other Chemicals in Environmental Matrices**

**4.04A.T-01 A Novel EI Source Optimized for Use with Hydrogen Carrier Gas in GC/MS and GC/MS/MS for Environmental Analyses**

*Angela Smith Henry[1], Craig Marvin[1], Tarun Anumol[1],* ***Amanda McQuay[1]****, Laura Miles[2], Anastasia Alekseyevna Andrianova[1] and Bruce D. Quimby[1], (1) Agilent Technologies, Inc., (2) Markes International Ltd, United*

*Kingdom*

Switching to hydrogen for gas chromatography (GC) and GC/MS analyses is a frequently discussed topic as helium price and availability concerns have increased in recent years. Since hydrogen is a reactive gas, hydrogenation and dechlorination reactions can and do occur in the mass spectrometer electron ionization (EI) source. These reactions can make applying hydrogen carrier gas to EPA methods, like 8270, difficult due to altered mass spectral ion ratios, spectral infidelity, and peak tailing. The development of a new and novel EI source to address these hydrogen-related issues improves performance with hydrogen carrier gas in GC/MS and GC/MS/MS analysis, specifically EPA method 8270(E), TO-15 and volatile organic compound analyses. The novel EI source also introduces the ability to shift to more efficient columns and a faster analysis time, while retaining mass spectra fidelity and reducing the need to re-develop libraries, quantitative methods, or multiple-reaction monitoring transitions. In the presentation, several examples will be shown demonstrating full method compliance, increased productivity, optimal peak shapes and the absence of any in-source reactions for a number of environmental standard methods, when using hydrogen carrier gas and modern GC/MS systems.

**4.04A.T-02 Unregulated Organic Chemicals in Biosolids: Prioritization, Fate and Risk Evaluation for Land Applications**

***Nicole M. Dennis****, Qingyang Shi and Jay Gan, University of California, Riverside*

The land application of biosolids (i.e., treated sewage sludge) offers multiple benefits over the hazards of landfilling and incineration and improves soil health and plant nutrition, leading to a circular economy. Biosolids have been recognized in the United States for nearly 50 years as a highly underutilized resource for agricultural purposes. However, biosolids contain numerous unregulated organic chemicals (UOCs) leading to concerns that may impede a positive public perception of the benefits gained by land application and hamper expansion of its beneficial use. Therefore, a critical research need exists to develop methods of chemical analysis and determine the occurrence, fate, and transport of UOCs in biosolids intended for land application, particularly those that may undergo offsite movement or bioaccumulation. It is further necessary to determine which UOCs in biosolids-amended soils may pose a high risk to human and ecosystem health. To address these imminent challenges, several universities were awarded EPA funding (EPA 84024501) to undertake a multi-year collaborative study utilizing a combination of field, laboratory, and modeling approaches. In our study, we will carry out laboratory studies to develop a rapid chemical assay using thin film passive samplers to predict the bioavailability of ‘priority’ UOCs in soil and further assess their bioaccumulation in earthworm tissue. Field validation data will be collected from passive samplers, earthworms, vegetables, and fruits to evaluate the bioavailability, uptake, and presence of the priority UOCs in the edible portions of food and feed crops and in earthworms. These results are expected to allow for rapid assessment of chemical bioavailability and prediction of bioaccumulation potential for an extremely diverse chemical mixture and contribute to a holistic human health and ecological risk assessment. Here we will present our study design and up to date research progress.

**4.04A.T-03 Concentrations of Quaternary Ammonium Compounds in Wastewater Treatment Plant Effluents during the COVID-19 Pandemic**

***Michael Gross****, Michelle L. Hladik, Paul M. Bradley, Kelly L. Smalling and Dana Kolpin, U.S. Geological Survey*

Quaternary ammonium compounds (QACs) are high production volume chemicals used in many commercial and household disinfection products. The U.S. Environmental Protection Agency (EPA) developed List N in March 2020, shortly following the emergence of the SARS-CoV-2 (COVID-19) pandemic. List N contains several disinfectants believed to kill all strains and variants of the COVID-19 virus. Currently, 606 products are listed, and 280 of these products use QACs as an active ingredient. It is hypothesized that the amount of QACs used has increased due to the pandemic. As a result, elevated levels of QACs are potentially entering wastewater treatment plants (WWTPs) and the environment. Increased concentrations of QACs gives rise to concerns of antibiotic resistance, disinfection byproducts, and toxicity to aquatic and soil organisms. Herein, a method was developed for the analysis of QACs in unfiltered, small volume (less than 40 mL) WWTP effluent samples. Target QACs included benzethonium, benzylalkyldimethyl ammonium compounds (BACs), ethylbenzylalkyldimethyl ammonium compounds (EBACs), and dialkyldimethyl ammonium compounds (DADMACs). Samples were processed via solid phase extraction using weak cation exchange (WCX) cartridges (3 cc, 60 mg) and analyzed via liquid chromatography tandem mass spectrometry (LC-MS/MS). Recoveries ranged 86.0-101.5%, with an average recovery of 92.7%. Effluents were analyzed from three separate WWTPs with sample collections from May to October of 2020. At least one QAC was detected in every sample with concentrations up to 1600 ng/L. BAC-$C_{14}$ was the most detected compound, found in 93% of effluent samples. BAC-$C_{12}$, BAC-$C_{16}$, EBAC-$C_{12}$ and EBAC-$C_{14}$ were all detected in greater than 80% of samples. No temporal patterns were observed with QACs with respect to the COVID-19 pandemic. WWTPs are highly effective at removing QACs from the municipal waste stream; however, concentrations were still detectable in effluent samples. Continued monitoring of QACs in WWTP waters and biosolids would improve understanding of their environmental fate and potential effects.

**4.04A.T-04 Determination of Synthetic Musk Compounds in Fish Fillets from Urban Streams and Rivers in the United States Using Gas Chromatography and Mass Spectrometry**

***Lantis Iyayi Osemwengie****, James M. Lazorchak and Angela Batt, U.S. Environmental Protection Agency*

In 2008-2009, the U.S. EPA and their partners collected fish during a probabilistic survey of 540 navigable water sampling sites in the contiguous U.S. under the National Rivers and Streams Assessment program (NRSA), a subset of which were 182 urban river sites. Fish fillets from urban river sites were analyzed for the occurrence and extent of selected contaminants of emerging concern (CEC), including pharmaceuticals and personal care products. Fish fillets were analyzed for synthetic musk compounds and nitro musk metabolites to estimate potential human exposures. Approximately 2 g of fish homogenate was surrogate fortified and accelerated solvent extracted with alumina in a stainless-steel extraction cell. Gel permeation chromatography was performed as a first cleanup step on each fish sample extract. The resultant extracts were then subjected to additional cleanup using amino propyl cartridges.  These samples were extracted and analyzed by GC/MS, using selected ion monitoring mode, for select synthetic musk compounds and nitro musk metabolites, i.e., nitro musk metabolites).  Upon analysis of 169 fish fillet samples, the polycyclic musks were found in varied concentrations, 756 ng/g to non-detect, while the nitro musk compounds were found either in low concentrations (<15 ng/g) (MDL) or non-detect. This study confirms the presence of personal care products, mainly Galaxolide and Tonalide, in urban national rivers and streams, and their bio-concentration by fish.

**4.04A.T-05 Targeted Chemical Analysis of sediments and water reveals site-specific contaminants of concern at Tijuana River Estuary**

***Flannery McLamb***[1]*, Damian Shea*[2] *and Goran Bozinovic*[3]*, (1) Boz Life Science Research and Teaching Institute, (2) Statera Environmental, Inc., (3) University of California, San Diego*

The Tijuana River Estuary (TRE) is a public health hazard and source of contention between the United States and Mexico for decades. The focus of this project was to characterize chemical pollution at four different TRE locations using targeted chemical analysis and a novel passive sampling device, the Composite Integrative Passive Sampler (CIPS), that quantitively accumulates polar and non-polar organic chemicals from water.

Sediment and water samples were collected, and CIPS deployed at four sites during dry weather and after a rain event, along the river from inland to coastal sites. Site 1 is near the US-Mexico border where the Tijuana River flows into the US; Site 2 is in the northern estuary, closest to the urban area of Imperial Beach; Sites 3 and 4 are in the southern arm of the estuary. Sediment samples and CIPS were analyzed by GCMS and LCMSMS for quantitative targeted analysis of >150 organic chemicals.

The highest concentrations of all chemical classes, in both sediment and CIPS, were at the US-Mexico border (Site 1), followed by Site 2 near Imperial Beach. Site 1 near the US-Mexico border that had 15-fold higher PAH than Sites 2 and 4. All sites exhibited a mixture of petrogenic and pyrogenic PAH, but the relative increase in

PAH at Site 3 was driven by an increase in pyrogenic PAH. Current use pesticides were dominated by pyrethroids and EPTC, while the Organochlorine pesticides were dominated by chlordanes, dieldrin, DDTs; PCBs were found only in the sediment and not in the CIPS, and concentrations were highest at Site 1, but in the low ng/g range. Phthalates were only found at Site 1 and in both the sediment and CIPS, with relatively high bis(2-ethylhexyl) phthalate.

CIPS accumulated nearly every chemical that was found in sediment and many water-soluble chemicals not detected in sediment. The presence of haloacetic acids in the CIPS at Site 1 near the border, but not at any other sites or in any sediment, suggesting a significant source of disinfection byproducts from treated sewage from Tijuana across the border.

This study provides positive identification and quantitative concentrations for organic pollutants in TRE sediments and water. There is a clear indication of a very significant source of all classes of chemicals coming across the border, with phthalates and haloacetic acids likely only coming from Tijuana, while other classes of chemicals have a significant input from Mexico superimposed on other smaller sources near the TRE.

**4.04A.T-06 Analytical Methods for Assessing Exposure to Flourine-Free Firefighting Foams: Implications for Determining Tissue-Specific Toxicity Reference Values**

***Farzana Hossain**[1], Anna Sophia Longwell[1], Seenivasan Subbiah[1], Jamie Suski[2] and Todd Anderson[1], (1) Texas Tech University, (2) EA Engineering, Science, and Technology, Inc., PBC*

Fluorine-free chemical constituents have been proposed for use in firefighting foams in an effort to reduce the potential negative environmental impacts of per- and poly-fluoroalkyl substances (PFAS). While there are potentially fewer issues with these new fluorine-free foams in terms of persistence, it is prudent to also evaluate their potential ecotoxicity. To do so requires analytical methods to chemically verify dosing solutions, exposure concentrations, whole body and tissue burdens, for example. Many of the foams are proprietary, so SDS sheets do not necessarily contain detailed information on specific chemical constituents and/or concentrations. Other than water, the main constituents of many fluorine-free firefighting foams are hexylene glycol, sodium dodecyl sulfate (SDS), and diethylene glycol monobutyl ether. We developed analytical methods for these constituents and others to use in support of ongoing avian chronic toxicity tests in our laboratory on several fluorine-free foams. Marker constituents that we identified using SDS sheets and LC-MS analysis were used to verify foam concentrations in drinking water exposures to adult quail breeding pairs; coupled with water consumption data, these measured concentrations were used to determine average daily intake (ADI). In addition, liver tissue samples will be collected from adults and 21-d chicks and analyzed for marker constituents in the foams to determine tissue burdens. These residue analyses will be used along with treatment-related survival, growth, and reproductive endpoints to derive tissue-specific Toxicity Reference Values (TRVs).

**4.04A.T-07 Overcoming the Analytical Challenge of Cationic Polar Pesticides**

***Carl Fisher**, Thermo Fisher Scientific*

Recent developments in the analysis of anionic polar pesticides have led to an increase in testing and regulation in various environmental matrices. However, developments in the analysis of cationic polar pesticides have lagged their anionic counterparts, primarily because of the analytical challenges. Advances in sample preparation combined with IC-MS/MS have successfully demonstrated the applicability for the analysis of anionic polar pesticides, particularly glyphosate. However, for cationic polar pesticides, previous work has demonstrated an inability to resolve the Diquat / Paraquat peak pair. This combined with their similar m/z ratios makes it difficult to use IC-MS/MS methods for this application. Cationic polar pesticides such as chlormequat, diquat, mepiquat and paraquat may be present, but may not be included in pesticide monitoring programs due to the difficulty in the determination of these target analytes using generic multi-residue methods.

To address this analytical challenge, a cationic exchange column was developed to baseline resolve the four key quaternary polar pesticides: chlormequat, diquat, mepiquat and paraquat. This work demonstrates a new IC-MS/MS method to identify and quantify low concentrations of quaternary amine cationic polar pesticides. In this presentation, the IC-MS/MS method will be discussed and its applicability for determination of chlormequat, diquat, mepiquat and paraquat will be demonstrated.

**4.04A.T-08 Do organic dietary interventions offer significant ameliorative benefits for human exposure to emerging pesticides? (Laying the emphasis on neonicotinoids)**

***Collins Nimako**, Anri Hirai, Takahiro Ichise, Shouta M.M. Nakayama, Mayumi Ishizuka and Yoshinori Ikenaka, Hokkaido University, Japan*

Neonicotinoid insecticides (NNIs) are a popular class of insecticides used in various pest management regimens worldwide. Emerging biomonitoring studies continuously report high exposure rates of NNIs in human populations, yet there is no validated countermeasure for combating the potential health implications of NNIs in human populations. This study assessed if compliance with organic dietary protocols offers significant ameliorative benefits in NNI exposures within human populations. About 103 organic (n=42) and conventional (n=61) tea leaves were sampled from grocery stores in Japan. Then, NNI residues were quantified in the tea leaves using LC-ESI/MS/MS. Subsequently, 103 Japanese volunteers were recruited and requested to adopt strict organic or conventional dietary interventions for at least 5 continuous days. After the interventions, urinary exposure rates of NNIs were determined in the population using LC-ESI/MS/MS. In both organic and conventional tea leaves, 8 NNI compounds were detected; and the detection frequencies (%Dfs) were in the decreasing order; thiacloprid (84.47%) > dinotefuran (74.76%) > imidacloprid (69.90%) ≈ clothianidin (69.90%) > dm-acetamiprid (63.11%) > thiamethoxam (58.25%) > acetamiprid (4.85%) > nitenpyram (1.94%). The %Dfs of NNIs were relatively lower in organic tea leaves than in conventional tea leaves. Various percentile concentrations of NNIs were far lower in organic tea leaves compared to conventional tea leaves. The daily intakes (EDIs) of NNIs through tea consumption were also lower for organic tea leaves than the conventional tea leaves. In the urinary analysis, 8 NNIs were detected in both organic- and conventional-diet consumers; with a decreasing %Df pattern; desmethyl-acetamiprid (64.96%) > dinotefuran (52.12%), imidacloprid (39.61%) > clothianidin (33.95%) > thiamethoxam (28.51%) > acetamiprid (12.62%) > nitenpyram (5.33%) > thiacloprid (2.83%). The %Df of NNIs in the organic diet consumers were lower than those of the conventional diet consumers. The mean and median cumulative levels of NNIs were significantly lower in the organic diet groups compared to the conventional diet group ($p < 0.0001$). Relative to the conventional dietary group, the organic dietary intervention showed drastic reductions in NNI EDIs. These ultimately suggested that strict compliance with organic dietary interventions may offer optimum ameliorative outcomes on NNI exposure rates in the human populations.

**4.04B Analysis of Pharmaceuticals, Pesticides, and Other Chemicals in Environmental Matrices**

**4.04B.T-01 Concentrations Of Chemicals Of Emerging Concern Are Mediated By Seasonal Hydrodynamics In An Offshore Marine Environment**

***Anna Robuck**[1], Michaela Cashman[2], Mark Cantwell[2], Michael Thompson[3], Christine Gardiner[4], Julia Sullivan[5], David Wiley[3,6], Pete DeCola[3] and Rainer Lohmann[4], (1) Icahn School of Medicine at Mount Sinai, (2) U.S. Environmental Protection Agency, (3) National Oceanic and Atmospheric Administration (NOAA), (4) University of Rhode Island, (5) Oak Ridge Associated Universities, (6) Stellwagen Bank National Marine Sanctuary*

Many contaminants of emerging concern (CECs), including pharmaceuticals and personal care products, are distinct from legacy contaminants in their ability to travel readily in aqueous matrices, resulting in the common occurrence of CECs in aquatic system receiving point and nonpoint source waste streams. However, little data currently describes CEC concentrations in marine ecosystems. Here we measured 17 pharmaceuticals, caffeine, sucralose, and 25 per- and polyfluoroalkyl substances (PFAS) surrounding a major wastewater discharge tunnel into Massachusetts Bay, USA. Water grabs were collected from surface and bottom water compartments during both stratified and unstratified water column conditions. Samples were analyzed for CECs using liquid chromatography coupled to tandem mass spectrometry. 10 of 17 pharmaceuticals, sucralose, caffeine, and 9 PFAS were detected in surface and bottom water at low (>40 ng/L) to very low concentrations (>2ng/L). Concentrations were proportional to proximity to the point source discharge, with the highest concentrations observed directly south of the outfall in accordance with regional circulation patterns. Stratified conditions mediated occurrence of CECs, with increased bottom water CEC concentrations during stratified conditions and increased surface water CEC concentrations during unstratified conditions. Concentrations of all analytes in both surface and bottom water were generally higher in winter, likely related to reduced biological degradation and uptake. This work highlights the potential for pharmaceuticals and other CECs to persist in marine environments, and underscores the need for further evaluation of potential impacts of these compounds in marine biota subject to chronic, low-level exposures.

**4.04B.T-02 Temporal and Spatial trends of Perfluoroalkyl Acids in Seawater in Arctic Canada**
***Amila O. De Silva***[1], *Liisa Jantunen*[1], *Sarah Bernstein*[1,2], *Christine Spencer*[1], *Peter Amarualik*[3], *Jane Kirk*[1] *and Derek Muir*[1], *(1) Environment and Climate Change Canada, Canada, (2)University of Toronto, Canada, (3) Resolute Bay, Canada*
Per and polyfluoroalkyl substances (PFAS) are under intense scrutiny for their environmental persistence including those that degrade to the ultimately persistent perfluoroalkyl acids (PFAA). Within the broad category of PFAA, we measure perfluoroalkyl carboxylic acids (PFCA) and perfluoroalkyl sulfonic acids (PFSA) in the Canadian Arctic Ocean. Since 2005, we have been sampling seawater from the Barrow Strait near the community of Resolute Bay in Nunavut Canada (74.59 N -94.91 W). For the majority of the chain lengths (PFBA, PFHxA, PFOA, PFNA, and PFOS) we note a decline in concentration over the past decade. Under-ice depth profiles from 0 to 100 m, sampled in late May show an inverse correlation of PFAA concentrations with major ions – Na, K, Mg and K. This suggests surface inputs of PFAA, likely through snow and ice melt. Our most recent analysis from 2021, show that total PFAA concentrations do not exceed 2.0 ng $L^{-1}$ in Barrow Strait. Spatial sampling was made possible as part of ArcticNet expeditions on the research vessels (R/V) CCGS Amundsen, the CCGS Wilfrid Laurier, and the R/V William Kennedy from 2017-19. Seawater samples were collected in the Beaufort Sea, Amundsen Gulf, Queen Maud Gulf, Hudson Bay, and Davis Strait. Further north, samples were collected from Barrow Strait and northern Baffin Bay. In surface water samples, median total (Σ) PFAA was 1.4 ng $L^{-1}$ and lowest concentration was in northern Baffin Bay. In September 2019, Baker Lake and its tributary the Thelon River at 120 km and 80 km, respectively from Hudson Bay were sampled. The lake and river had the highest concentrations of Σ PFAA, approximately 6.0 and 7.4 ng $L^{-1}$ (salinity: 0.11 to 0.27 PSU). In Hudson Bay at Chesterfield Inlet near the outlet of Thelon River, Σ PFAA concentrations dropped to 2.0 ng $L^{-1}$ (salinity 28 PSU). Spatially, the lowest concentrations were in western Arctic including the Amundsen Gulf and Beaufort Sea (0.3 to 0.5 ng $L^{-1}$ ΣPFAA). Northern and eastern Hudson Bay were in the 0.5 to 1.0 ng $L^{-1}$ ΣPFAA range. Our findings highlight that though PFAA concentrations are declining, their persistence and mobility in the aquatic environment contribute to their ubiquitous presence in the Arctic in the present day.

**4.04B.T-03 Identifying Advective Flow of Contaminants Across Sediment-Water Interface Using Polyethylene Passive Samplers**
***Alanna Sparagna***[1], *Loretta Fernandez*[1], *Jiakai Yuan*[1], *Carlos E. Ruiz*[2] *and Philip Gidley*[2], *(1) Northeastern University, (2) U.S. Army Engineer Research and Development Center*
Contaminated sediments can be found in every major waterway within the United States. Assessing the environmental risk of these contaminated sediments related to hydrophobic organic contaminants such as polychlorinated biphenyls (PCBs) includes understanding their rate of transport between sediments and the water column. In situ passive sampling methods have been used to estimate the diffusive transport of PCBs across the sediment-water interface. Few methods are available to detect advective forms of transport including bioirrigation and hyporheic water exchange. This work sought to validate a method for using polyethylene

passive (PE) samplers deployed across the sediment-water interface to detect advective flow and the related contaminant transport. In addition, methods for using performance reference compounds (PRCs) to determine freely-dissolved sediment porewater concentrations ($C_{free}$) in systems with porewater flow were tested.

In order to examine the transport of contaminants in systems that had advective flow across the sediment-water interface, flow-through columns containing contaminated sediments were set up at the Engineering Research and Development Center (ERDC), which allowed us to insert PE strip samplers containing PRCs across the interface while clean water was pumped from the bottom of the column up at various speeds. Overflow waters were collected and extracted to determine total PCB transport from the sediment. After two months, PE strips were removed and extracted to determine the mass of PCBs taken up by samplers and the mass of PRCs lost from samplers. These values were used in conjunction with published PE passive sampling methods to determine the fractional equilibration ($f_{eq}$) achieved in each column and to calculate $C_{free}$.

As expected, higher flow rates through the columns corresponded with higher nominal $f_{eq}$ values for four PRCs ($^{13}$C-PCB-8,-28, -52, -118). Application of these $f_{eq}$ allowed for the consistent determination of $C_{free}$ regardless of flow rate for PCB congeners that match the PRCs. The contaminant fluxes across the sediment-water interface determined by extracting overflow water were consistently within a factor of three of those determined using passive samplers and known Darcy flowrates. This work further presents a method for using sampler-determined $f_{eq}$ and laboratory-determined $K_d$ to estimate advective flow rates in column experiments.

**4.04B.T-04 Assessment of Anticoagulant Rodenticides in Terrestrial and Aquatic Non-target Organisms - a Case Study of Switzerland**

***Carolin Riegraf*[1]**, *Daniel Olbrich[2], Petra Kunz[3] and Etienne Vermeirssen[2], (1) Oekotoxzentrum Eawag-EPFL, Switzerland, (2) Swiss Centre for Applied Ecotoxicology, Switzerland, (3) Federal Office for the Environment Switzerland, Switzerland*

The global use of anticoagulant rodenticides (ARs) for rodent control has led to their presence in the aquatic and terrestrial environment. ARs can enter the environment through in- and outdoor surface applications such as in agriculture, industrial or residential areas and through baits installed in sewer systems or on landfill sites. Due to the intelligent nature of rodents, the effect of applied and consumed ARs needs to be delayed to prevent bait avoidance. However, delayed effects require persistent, toxic and bioaccumulative AR compound properties, which in turn pose a risk to the environment. This negative impact has been observed to lead to secondary poisoning of non-target organisms such as birds of prey or foxes. Despite the documented impacts on non-target organisms, only a limited number of adequate AR-alternatives are available for rodent control to date. To obtain a better insight into the application, distribution and occurrence in non-target biota, we consulted cantonal and communal authorities and pest controllers by means of interviews and questionnaires to make a nationwide estimate of the applied AR quantities in Switzerland. In addition, we conducted a screening of AR residuals in non-target organisms. Results of interviews and questionnaires showed that a central and systematic collection of data on amounts of ARs used is missing, as there are no reporting requirements to date. Hence, an estimation of AR quantities applied in Switzerland is currently challenging. Sensitive analytical methods (LC-MS/MS) for the detection of seven authorized ARs (Brodifacoum, Bromadiolone, Coumatetralyl, Difenacoum, Difethialone, Flocoumafen, Warfarin) in liver samples were successfully established. Livers of foxes (healthy, shot during regular hunting) provided by the Institute of Parasitology (Vetsuisse Faculty, University of Zurich), birds of prey and hedgehogs (found dead or sick and died during care) provided by a bird-of-prey rehabilitation center (Greifvogelstation Berg am Irchel) with support from Vetsuisse Faculty and SWILD, respectively, and fish (healthy) provided by cantonal authorities were analyzed. A comparison with data collated from literature showed similar exposure in biota from surrounding countries. To conclude, the AR burden in the Swiss environment might be higher than anticipated and a thorough investigation of AR residues in different non-target organisms is needed as a basis to propose an adequate risk management.

**4.04B.T-06 Analyzing the effect of the differences in PM2.5 Composition, Oxidative Potential, and Developmental Toxicity Collected Across Months at Locations Throughout Tennessee**

***Voke Tonia Aminone**, Anna Tu and Courtney Roper, University of Mississippi*

Air pollution is considered the most significant environmental risk factor to human health. Fine particulate matter ($PM_{2.5}$) is a complex mixture in the air that varies based on meteorology and location. $PM_{2.5}$ can cause health outcomes via oxidative stress, including impacts on development. Black carbon is a component of $PM_{2.5}$ that is also associated with causing developmental effects. Zebrafish are a developmental model of human disease. The goal of our research was to determine if developmental exposure to $PM_{2.5}$ from varying locations and months alters zebrafish development. The Tennessee Department of Environment and Conservation (TDEC) donated $PM_{2.5}$ filters collected in September and November from 4 locations (Loudon, Harriman, Dyersburg and Jackson, n=14/location). Prior to extraction, black carbon concentrations were measured. $PM_{2.5}$ was extracted from filters by sonication in methanol. Aliquots of the daily extracted $PM_{2.5}$ were removed to assess oxidative potential using the dithiothreitol (DTT) assay. The pooled $PM_{2.5}$ samples (based on month /location) and controls (vehicle, field blank), were then used to expose zebrafish (n=33) from 6 hours post fertilization to 5 days post fertilization for developmental toxicity analysis. The mortality, morphology and behavioral changes were measured and comparisons were made between groups based on the sampling location and month. Average black carbon concentrations ranged in September (1.38 to 2.11$\mu g/m^3$) and November (1.11 to 2.47 $\mu g/m^3$) as did $PM_{2.5}$ concentrations in September (7.57 to 8.22 $\mu g/m^3$) and November (7.02 to 12.3 $\mu g/m^3$). Developmental toxicity varied based on the location and month that $PM_{2.5}$ was collected and we saw more malformations in November, particularly for one location compared to the other locations (survival/hatch rates were above 80% for all treatment groups except Loudon in November). Significant positive correlations were observed between oxidative potential and $PM_{2.5}$/BC concentrations in both months. Analysis is underway for zebrafish behavioral assessments and the impacts of meteorological parameters on $PM_{2.5}$/BC concentrations. The findings from this research will help establish if the environmental contaminants and oxidative potential differ based on locations and months, their developmental and behavioral effects in zebrafish, the role the contaminants play in these responses, and if they are affected by meteorological parameters.

**4.04B.T-07 Discussion 1 of 2 - Analysis of pharmaceuticals, pesticides, and other chemicals in environmental matrices**

***Yu Ye**[1], Nicole M. Dennis[2] and Wesley S. Hunter[1], (1) U.S. Food and Drug Administration, (2) Texas Tech University*

The discussion slots at the end of the session aim to give participants an opportunity to speak about the analytical challenges in their studies. Others may be able to exchange ideas about solutions and alternative approaches.

**4.04B.T-08 Discussion 2 of 2 - Analysis of pharmaceuticals, pesticides, and other chemicals in environmental matrices**

***Wesley S. Hunter**, U.S. Food and Drug Administration*

The discussion slots at the end of the session aim to give participants an opportunity to speak about the analytical challenges in their studies. Others may be able to exchange ideas about solutions and alternative approaches.

**4.05.P Approaches, Insights and Examples in Reconstructing Historical Pollution Records Throughout the Holocene Epoch**

**4.05.P-Tu085 Historical Environmental Health Changes of a Superfund Site in Puerto Rico**

*Michael Martinez-Colon[1], **Adebayo S. Solanke**[1], Benjamin Ross[1] and Roberto Viqueira-Rios[2], (1) Florida A&M University, (2) Protectores de Cuencas, Inc., Puerto Rico*

In September 2021, the United States Environmental Protection Agency proposed adding the Ochoa Fertilizer Co. site adjacent to Guánica Bay to the National Priorities List of hazardous waste sites as a Superfund site. Pollutants such as polychlorinated biphenyls (PCBs), nickel, and chromium (among others) have been found in the bay's waters, surface sediments, fish/coral tissue, and human blood (in the case of PCBs). It has been reported that this bay contains the second largest concentration (4,000–140,000 ng/g) of PCBs in the world. To assess the historical changes in environmental health of the bay, trace metals were analyzed from a 52 cm sediment core collected in October 2021. The water column at the time of collection was stratified with respect to temperature, pH, dissolved oxygen (DO), and salinity at a depth of 1.5 m. Most importantly, between 1.4–2.2 m the water is hypoxic (DO: 3.0–0.5 mg/L) and at depths greater than 2.2 m the water is anoxic (< 0.5 mg/L). This is critical because redox conditions play an integral role in the fate and transport of pollutants. A noticeable change in sedimentation was observed at a core depth of 34 cm ($^{14}C$ dating is pending) where total organic carbon, carbon-to-nitrogen molar ratios, and mud-sized sediments begin to decline towards the core top. Similarly trace metals like Cu-Pb and Ni-Hg-U decrease and increase in concentrations respectively. All these trace metals exceeded the Effect Range Median values stipulated by EPA, strongly suggesting that the flora and fauna have been historically impacted. A two- to 46-fold increase in Hg concentrations (max= 31 mg/kg at core top) is observed < 20 cm depth, which is indicative of severe pollution. More interestingly are the pseudo-total U concentrations which ranged from 128–207 mg/kg, although it is uncertain at the moment whether its historical source is allochthonous or autochthonous. Benthic foraminifera are currently being hand-picked from sediment samples for application as a proxy for environmental health. The *Ammonia-Elphidium* and Foraminiferal Stress indices will shed information on the level of pollution impact.

### 4.05.P-Tu086 Bird Feathers as a Method of Reconstructing Historical Exposure to Per- and Polyfluoroalkyl Substances (PFAS)

***Matthew Badia*** *and Greg Foster, George Mason University*

Per- and Polyfluoroalkyl Substances (PFAS) are of growing concern for their potential adverse environmental and human health effects. PFAS compounds demonstrate remarkable environmental stability and are globally dispersed. These compounds have been detected in a variety of biological samples, including bird feathers, which have proven to be a reliable indicator for the bioaccumulation of heavy metals and persistent organic pollutants. Our study aims to establish a chronology of PFAS exposure in birds from the Amazonian and the South American Atlantic Rainforest and determine if the rainforest bird populations are impacted by PFAS. Feathers, composed mainly of keratin, accumulate PFAS, which has a documented affinity for proteins. Feather samples were sourced from museum collections at the Smithsonian Institute and the University of Sao Paulo, Brazil. Samples were selected among fish-eating, insect-eating, and fruit-eating trophic guilds. Feathers collected prior to 1935, representing pre-exposure conditions, after 1935, representing post-exposure conditions, and the 2000s, representing current conditions, were used to establish a chronology of PFAS pollution in the Amazonian and Atlantic Rainforest. Feathers are ground to a fine powder, extracted using 0.3% methanolic ammonium hydroxide, and analyzed for 24 PFAS compounds. A Shimadzu Triple-Quadrupole Liquid Chromatography-Mass Spectrometer in Multiple Reactions Monitoring (MRM) mode was used to determine PFAS concentrations. PFAS concentrations are compared by species, feeding guild, age, and collection location. Bird specimen samples have been analyzed, and preliminary results show PFAS concentrations vary through time in some species. The results of this study have significance in determining the pervasiveness of PFAS in both developed and undeveloped regions of South American rainforests. Other researchers will use the chronology of PFAS introduction into the rainforests established in this study to determine if PFAS is a contributing factor in declining biodiversity.

### 4.05.V Approaches, Insights and Examples in Reconstructing Historical Pollution Records Throughout the Holocene Epoch

**4.05.V-01 Using Museum-Archived Myctophids to Model Their Role as Vectors of Microplastics to the Mesopelagic Food Web: a 60-Year Timescale**

***Olivia Cassidy Boisen**, Susanne M. Brander and Scott Heppell, Oregon State University*

Due to their small size, microplastics (< 5mm) in the marine environment are bioavailable to pelagic fishes and have been shown to cause a suite of harmful effects when consumed. With an estimated biomass of one to ten billion metric tons, the largest migratory mass on the planet occurs nightly by fishes from the family Myctophidae (lanternfishes) and associated communities. Known as diel vertical migration, these small-bodied fishes travel further than half a kilometer under the cover of night to feed on zooplankton at the ocean's surface. Buoyant microplastics are also concentrated at the surface and are presumed to be mistakenly ingested during feeding events. When the myctophids return to the deep, they are key prey for many mesopelagic predators; and thus act as a massive biological carbon, nutrient, and potentially microplastic pump. Since these predators are less likely to encounter microplastics at the depth they occupy, myctophids may serve as an important coupling vector for transporting microplastics deep below the surface. Myctophids (*Tarletonbeania crenularis*, *Diaphus theta*, and *Stenobrachius leucopsarus*) are ubiquitous in museum collections and their preservation provides a unique opportunity to investigate the spatial and temporal scope of microplastic internalization in the California Current Large Marine Ecosystem as global plastic production has increased over time. Particles isolated from the digestive tracts of archived myctophids will be used to evaluate the presence, magnitude, and change in microplastic composition from the 1960s to the present. We hypothesize that plastic ingestion will have increased dramatically over the time series and that the types of microplastics found in myctophids will be representative of plastics manufactured globally with a time lag of several years, as larger plastics take time to degrade into microplastics. In addition to quantifying historic contamination levels, the slope generated from this time series will indicate the trajectory of microplastic occurrence in mesopelagic food webs, allowing for predictive power of future debris scenarios. Broadly, mapping the extent of microplastic intake by myctophids over time will help fishery managers and healthcare officials establish acceptable levels of microplastics in marine ecosystems.

**4.06.P Biodegradation of Polymers in the Environment**

**4.06.P-We105 Gel Permeation Chromatography (GPC) Analysis of Modified Cellulose Polymers During OECD 302B Biodegradation Test**

***Carrie E. Jantzen**, Nigel Crabtree, Alan Fernyhough, Ryan Hamilton, Saiful Ahmed and Andrea Burron, Ashland*

The OECD Testing Guideline 302B (Zahn-Wellens/EMPA Test) is used to assess materials for classification as "inherently biodegradable". While such OECD study parameters and thresholds were initially designed to test small molecules, polymers have also been studied using this method as there are limited alternative assays available for polymers. Due to their larger sizes and complex structures, polymers, including natural based polymers, are less likely to pass the thresholds for biodegradation classification in this type of assay. In this study, three types of modified cellulose polymeric materials: hydroxypropylmethylcellulose (HPMC), hydroxyethylcellulose (HEC), and hydroxypropylcellulose (HPC) were assessed for biodegradation through an extended (60 days) OECD 302B assay, with a concurrent GPC analysis at the following timepoints (days 0, 7, 14, 21, 28, 42, and 60) to measure changes in average molecular weights and molecular weight distributions of the polymers after such exposures. At the 28- and 60- day timepoints, the percentage of biodegradation for all celluloses was below any classification OECD threshold (< 20%), however significantly decreased molecular weights, and shifts in the molecular weight distributions were clearly observed. These results indicate that although the OECD percent biodegradation metric appears to indicate little to no biodegradation is occurring, molecular weight changes and potential chemical alterations are observed throughout the course of the assay. Additional analytical studies, and/or potential modifications to assay parameters, may be necessary to understand the degradation of the cellulosic polymers more accurately during such OECD 302B type testing.

**4.06.P-We106 The Potentials of Two Plant Growth-Promoting Bacillus Species as Nanoplastic Biodegraders**

***Fatai Olabemiwo**, Frederick Cohan, Ama Hagan and Melanie Cham, Wesleyan University*

Nanoplastic waste in the environment is exponentially increasing due to the breakdown of macroplastics. When plastic breaks into tiny, nanoplastic particles, diverse consequences on organisms from microbes to vertebrates become possible. Numerous studies have investigated the harmful effects of nanoplastic particles on microbes. However, it is unclear whether the nanoplastic particles can be both beneficial and harmful to bacteria depending on the dosages, a toxicology phenomenon known as hormesis. Here, we investigated whether two known plant growth-promoting rhizobacteria (*Bacillus inaquosorum* and *B. velezensis*) can benefit from nanoplastic exposure. We exposed the bacteria strains of these two species to 25 nm polystyrene (PS) nanoplastics at various dosages and showed that the bacteria could utilize plastic as a sole carbon source at low dosages. A growth assay demonstrated that the bacteria growth was inhibited at low and high doses during short-term exposure. Despite these short-term inhibitions from plastic exposure, over a period of 24 hours, the bacteria grew to a higher density at a low dose and a lower density at a high dose compared to growth with no plastics. We also observed that the nanoplastics particles do not aggregate around the cell walls of the bacteria, and therefore, there is a weak interaction. Because these strains are able to utilize plastics, we conclude that they may be developed as nanoplastic bioremediators.

**4.06.P-We108 Aerobic Biodegradation of Polymers under Composting Conditions Using a Respirometer**

*Jean-Rene Thelusmond, **Yunzhou Chai**, Vurtice Albright, Matthew LeBaron, Jing Hu, Gary Kozerski, Nathalie Vallotton and Sue Marty, The Dow Chemical Company*

The biodegradability of cellulose, poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), polyvinyl acetate (PVAc), poly (vinyl alcohol) (PVOH), and walnut shell powder was evaluated in three compost sources. Carbon dioxide ($CO_2$) evolution, determined in an open flow respirometer, was used as a proxy for the biodegradability measurement based on a modified ASTM D5338-15. Another objective of this study was to evaluate the impact of the polymer biodegradation on the compost microbial structure using 16S rRNA amplicon sequencing. Before setting up the experiment, the physicochemical characteristics (e.g., moisture content, dry solids, volatile solids, total organic carbon, pH, C/N) of the compost were determined. The experimental set up consisted of 1-L glass bottles containing compost sieved through a 4 mm screen. Deionized water was used to adjust the moisture content in each composting reactor to about 60% before and after adding the designated test material, which was thoroughly mixed with the compost. The blend was transferred to 1-L glass reactors, capped and incubated at 58±2 °C. The airflow to each reactor was set at 250 mL/min. The $CO_2$ production in each reactor was recorded every 6.67 h. Biodegradation results from a preliminary experiment illustrate that cellulose biodegraded relatively fast in compost with over 90% converted to $CO_2$ within 45 days. Additional experiments are underway to determine the biodegradability of the above polymers in compost along with their potential effects on the microbial community structure.

**4.06.P-We109 Evaluating Polymer Biodegradation using Respirometry and Radiolabels**

***Ashley Wilcox**, Jennifer Menzies, Kathleen McDonough and Ken Casteel, Procter & Gamble*

Due to changes in regulatory requirements and continued development of novel chemistries there is a renewed focus on the development of biodegradable polymers. It is therefore important to evaluate and validate existing test methods (OECD 301B, OECD 301F, OECD 302B) for their use in assessing polymer biodegradation. Respirometric tests following the indirect measurements of $CO_2$ evolution or oxygen consumption are commonly used to assess test chemical mineralization. This poster will discuss recent research using different respirometric test systems and microbial communities from different environmental compartments to evaluate polymer mineralization. It will further discuss test system modifications needed to adequately assess biodegradation for different classes of polymers. We will also provide data from a study utilizing a radiolabeled polymer in an OECD 314B activated sludge die away simulation study to evaluate polymer biodegradation during wastewater treatment.

**4.06.P-We111 The Metabolism of the 6:2 Fluorotelomer Alcohol (FTOH) and its Role in Covalent Protein Modifications**

***Risha Minocha-Mckenney**, Amy Rand, Todd Harris and Keegan Harris, Carleton University, Canada*

Fluorotelomer alcohols (FTOHs) are found at relatively high levels in household products, indoor dust, and air. FTOHs have been proposed to cause toxicity in humans upon exposure, although the mechanism of action is uncertain. One hypothesis is that FTOHs metabolize to form bioactive metabolites. Furthermore, the formation of fluorotelomer aldehydes (FTALs) and fluorotelomer unsaturated aldehydes (FTUALs) may contribute to the observed toxicity through covalent protein modification and protein inactivation. Our preliminary research using human serum albumin as a model protein demonstrated that 6:2 FTUAL modified predominantly lysine residues as well as 19 arginine and histidine residues. However, specific endogenous proteins targeted by FTALs and/or FTUALs have not been elucidated. This project focused on identifying the specific protein(s) modified upon 6:2 FTOH exposure. Given the reactivity of these electrophilic aldehyde metabolites, we hypothesized that 6:2 FTAL and/or 6:2 FTUAL would act as a suicide inhibitor of the protein responsible for their formation. The 6:2 FTOH was exposed to human liver microsomes and purified recombinant proteins (CYP2A6, 2E1, and 3A4). Metabolism of 6:2 FTOH to the 6:2 FTAL and FTUAL was monitored using chemical derivatization with 2,4-dinitrophenylhydrazine (DNPH) and targeted LC-MS/MS analysis. Covalent protein modification by 6:2 FTAL and/or FTUAL was monitored using LC-MS/MS proteomic methods. Modified protein samples were processed by in-solution trypsin digestion prior to analysis. Results demonstrated that CYP2A6 is active towards 6:2 FTOH transformation to 6:2 FTAL and 6:2 FTUAL, and we propose that this enzyme is targeted for covalent modification. Finally, the kinetics of CYP2A6 inhibition will be monitored using fluorescence spectroscopy to determine the specific inhibition mechanism. Using a combination of analytical techniques, we can further elucidate the potential mechanism of action of 6:2 FTOH toxicity through its bioactivation.

**4.06.P-We112 Exploring Structure-Activity Relationships for Polymer Biodegradation**

*Joonrae Roger Kim, Jean-Rene Thelusmond, Vurtice Albright and **Yunzhou Chai**, The Dow Chemical Company*

Research on the environmental biodegradation of polymers has substantially increased recently due to growing demand for biodegradable polymers for certain applications. The biodegradability of a polymer depends to a large extent on the chemical structure of the polymer although environmental factors also play an important role. Quantitative structure-activity relationships (QSARs) have been well-established for the biodegradation of nonpolymeric organic chemicals, but not for the biodegradation of polymers. In this review paper, the authors explore structure-activity relationships (SARs) for biodegradability of polymers in various environmental compartments even though existing data are too scarce for the development of QSARs. In general, biodegradability is less for C-C chain polymers (polyolefins), and greater for polymers containing ester, ether, or amide bonds in their polymer chain. Under a univariate scenario, higher MW, higher crosslinking, lower water solubility, higher degree of substitution, and higher crystallinity may result in lower biodegradability of polymers. This review paper also stresses the need for better characterization of polymer structures used in biodegradation studies and consistent testing conditions for the ease of cross-comparison and quantitative modeling analysis.

**4.06.P-We113 Microbial Processes of Environmental Plastic Weathering and Biodegradation in Natural Systems**

*Melissa Duhaime[1], **Rachel N. Cable**[1], Jessica Coi[1], Agniva Bhaumik[1], Isabelle Montilla[1], Max Murray[1], Shuqing Zhang[1], Ting Lin[1], Piyush Thakre[2], Cristina Serrat[2], Yujing Tan[2], Jing Hu[2], David Meunier[2], Yuming Lai[2] and Zhan Chen[1], (1)University of Michigan, (2) The Dow Chemical Company*

PROBLEM: Plastics waste pervades in the environment is on track to continue its exponential increase, reflecting the global rate of plastics production. To effectively target major sources and pathways on a global scale, the question remains: What is the physical fate of plastic once in natural waterways? Our work is experimentally resolving the knowledge gaps regarding the fate of plastic debris in freshwater systems.

APPROACH: In both *in situ* and laboratory settings, we have studied UV and bio-degradation as individual processes, as well as their cumulative effects on polyolefin products designed for packaging. In our *in situ* work, using a combination of 16S rRNA gene amplicon sequencing and confocal microscopy, we have found that microbial colonization of plastics is influenced by UV aging and have determined the rates of biofilm growth in the environment, which is essential to the hydrodynamics that underlie plastic transport models. In the laboratory, we focus on the culturing and metabolic characterization of PE-degrading microbes. While decades of studies have reported microbes capable of weathering plastic surfaces, few have confirmed the microbial mineralization of plastics-derived carbon and fewer have resolved biodegradation mechanisms, let alone for environmentally relevant microbes and conditions. To address this, we have identified over 80 reported PE-degrading microbes. Of these, we have established a culture collection of 16 bacterial (n=10; 3 Phyla, 4 Classes, 6 Orders, 8 Families) and fungal (n=6; 3 Phyla, 5 Classes, 5 Orders, 6 Families) strains from a range of habitats (e.g., water, ocean, soil, wounds). We have used comparative genomics to identify shared metabolic pathways. These results inform the taxonomic and functional analysis of biofilms from plastics recovered from the environment (*in situ* work, above), bridging the gap between characterized single strain isolates and largely uncharacterized mixed microbial communities.

OUTLOOK: We will discuss how metabolic models of PE-degrading microbes and multi-species consortia can be used to predict the impact on genetically engineered improvements, identify growth factors needed by difficult to isolate microbes, and predict outcomes of different conditions in engineered plastics bioreactors. The knowledge gaps we address will improve our ability to model the environmental fate of plastics, engineer solutions, and design and recover polymers for circular economies.

## 4.07 Chemicals in Domestic and Industrial Wastewaters: Occurrence, Fate, and Use as Tracers

### 4.07.T-01 Investigation of PFAS in Wastewater Matrices to Inform Management

*Diana Lin, **Miguel Alexander Mendez** and Rebecca Sutton, San Francisco Estuary Institute*

Per- and polyfluoroalkyl substances (PFAS) are a diverse class of chemicals used broadly in consumer and industrial products that are receiving significant attention from scientists, communities, and regulators due to concerns about their persistence, bioaccumulation, and toxicity and impacts to human health and ecosystems. Strategies to mitigate PFAS require further understanding of the major sources and pathways leading to the release of PFAS to the environment. We evaluated the presence of PFAS in wastewater matrices in the San Francisco Bay region to inform management actions on PFAS. Wastewater influent, effluent, and biosolids were collected from a representative set of 16 publicly owned treatment works (POTWs) in the region to assess the presence of PFAS. Samples were analyzed by LC-MS/MS and 40 PFAS analytes were quantified by isotope dilution/internal standard quantification methods (a targeted method). Additionally, the presence of PFAS precursors in influent and biosolids were assessed by converting oxidizable PFAS to terminal PFAS in samples prior to analysis by LC-MS/MS (Total Oxidizable Precursors; TOP method). Follow-up investigations were conducted to evaluate the relative importance of wastewater from residential sources compared to industrial wastewater discharged to municipal facilities. Sum of PFAS measured using both targeted (minimum – maximum, 10 – 59 ng/L) and TOP methods (150 – 299 ng/L) agreed within an order of magnitude among municipal POTWs. The sum of PFAS measured using the TOP method was significantly higher than the sum of PFAS measured using the targeted method, which indicates a significant presence of PFAS precursors in wastewater. This study demonstrated a cost-effective and collaborative approach to investigating the major PFAS sources to receiving waters to inform management actions, and can be a model for other regions.

**4.07.T-02 Investigating the Enantiospecific Behaviour of Chiral Anti-depressants in Wastewater and Surface Water in Grand River, Ontario, Canada**

***Sondus Jamal***, *Leslie Bragg, Wayne Parker and Mark R. Servos, University of Waterloo, Canada*

Antidepressants used in urban settings are increasingly being detected in wastewaters and receiving waters at trace levels (i.e. µg/L to ng/L). They are considered pseudo-persistent due to their constant introduction into the aquatic environment via treated wastewater discharges. Antidepressants such as venlafaxine (VEN) and fluoxetine (FLX) are chiral and are typically marketed as racemates. The enantiospecific behaviour of these compounds, and their active metabolites desvenlafaxine (desVEN) and norfluoxetine (NFLX) respectively has been studied in the context of their pharmacokinetics and pharmacodynamics. However, limited work has been done in environmental matrices. The current research aims to investigate the enantiospecific behaviour of VEN, desVEN, FLX, and NFLX in wastewater treatment and in surface water using the Grand River, Ontario, Canada as the platform. Monthly sampling from Oct 2020 till Aug 2021 was carried out at three wastewater treatment plants (WWTPs) (i.e. 24-h composites of raw and treated wastewater were collected over 3 consecutive days each month). Seasonal sampling was carried out at 15 river sites in the fall of 2019, and at 26 river sites in the fall of 2020 and summer of 2021. Samples were collected in triplicates and pre-treated prior to solid phase extraction then analyzed using LC-MS/MS with a chiral column. Raw wastewaters were consistently enriched in the R- enantiomer of desVEN; however, the extent of this enrichment decreased through each of the wastewater treatment plants. The results indicate that transformation processes within the WWTPs were enantioselective. Within the river reaches, directly downstream of WWTP discharge points, desVEN was generally S-enriched (except downstream of Kitchener in Fall 2020), and the extent of the enrichment increased with distance away from the discharge. Hence, it appears that the in-stream degradation processes are enantioselective of R-desVEN resulting in S-enrichment of desVEN in the river environment. The different compounds exhibited varying enantiomeric distributions, and compositions in river and wastewater samples. The levels of R and S enrichment varied along the river, and downstream of WWTPs. The outcomes of this research will support water and wastewater managers to better assess and manage the potential environmental risks of chiral pharmaceuticals.

**4.07.T-03 Increased Brine Salt Concentrations in Pennsylvania Groundwater Indicate Potential Wastewater Impacts During Unconventional Oil & Gas Development**

***Samuel Shaheen***[1], *Tao Wen*[2] *and Susan Brantley*[1], *(1) The Pennsylvania State University, (2) Syracuse University*

The recent boom in natural gas production from shale formations via unconventional oil & gas development (UOGD) has advanced U.S. energy independence, but sparked concerns regarding environmental and human health impacts. For example, produced and flowback waters, which are generated throughout the production life of an unconventional well, contain high concentrations of salts, organics, and radioactive species. Natural gas production from the Marcellus Shale in Pennsylvania has resulted in occasional spills or leaks of such UOGD wastewaters. However, the extent to which wastewater spills or leaks have contaminated shallow groundwater, a major source of drinking water in rural Pennsylvania, is poorly understood. In this study, we investigated potential impacts of UOGD on groundwater quality using a 7,000-sample dataset of groundwater analyses from Beaver, Washington, and Greene counties in southwestern Pennsylvania, a hotbed of drilling in the Marcellus Shale. We identified statistically significant increases in chloride, barium, and strontium concentrations, all of which are present at high concentrations in UOGD produced water, associated with increased proximity to or density of UOGD. Using a geospatial data mining technique, we discovered these correlations are likely the result of smaller "hotspots" of contamination, rather than evenly distributed contamination across the region. If produced waters are indeed reaching groundwater in these locations, our calculations suggest that concentrations of several toxic trace elements such as arsenic and thallium may approach or exceed regulatory limits, posing a potential threat to human health.

**4.07.T-04 Water recovery and reuse from unconventional produced waters using membrane distillation**
***Radisav Vidic**, Ritesh Pawar and Zhewei Zhang, University of Pittsburgh*
Produced water from unconventional oil and gas reservoirs contains high concentrations of organic and inorganic chemicals, bacteria, sand or mud, oil and grease, Naturally Occurring Radioactive Materials (NORM) and is not amenable to conventional wastewater treatment processes. More than 90% of produced water is disposed in Class II injection wells and is permanently lost from the hydrologic cycle. Membrane Distillation (MD) is a unique treatment technology that can potentially be used for such complex wastewaters because it employs the vapor pressure difference across the membrane as a driving force for separation, which is not significantly affected by the salinity. The presence of high concentrations of organics can be a challenge for MD technology because some compounds can cause membrane fouling or wetting, thereby reducing separation efficiency.

Laboratory-scale experiments with actual produced water from Permian Basin (TX), Bakken Formation (ND) and Marcellus Shale play (PA) were used to assess MD performance and establish the range of operating parameters that would ensure steady-state operation at maximum water recovery. The TDS content of these produced waters varied from 100 g/L to 180 g/L and hence, the achievable water recoveries were different. Long-term tests showed that 50% water recovery is achievable for produced water from Permian Basin. However, an increase in permeate conductivity with water recovery was observed and further investigation revealed that the ammonia present in the produced water is transferred to the permeate side where protonation results in conductivity increase. Ongoing studies are designed to evaluate the nature of organics in the feed and the permeate and their impact on MD performance.  In addition, whole effluent toxicity testing with different permeates will reveal the potential for beneficial reuse of this recovered water.

**4.07.T-05 Uncovering Behavior Patterns of Trace Organic Contaminants in Nitrogen Removing Biofilters Using High Resolution Mass Spectrometry**
***Rachel Smolinski** and Carrie A. McDonough, Stony Brook University*
Onsite wastewater treatment systems pollute groundwater, surface water, and public drinking water resources with nutrients and trace organic contaminants (TOrCs). In Suffolk County, New York, over 360,000 residences are served by onsite wastewater treatment systems resulting in widespread pollution and consequent push for improved infrastructure. Nitrogen removing biofilters (NRBs) are promising alternative onsite wastewater treatment systems designed to mitigate nutrient pollution and have shown promise in removing a suite of TOrCs through targeted screening approaches. In the pressing interest of protecting clean water resources and understanding the mechanisms TOrC behavior in NRBs, nontarget analysis workflows are implemented in this work to identify TOrCs overlooked by traditional targeted screening techniques while defining TOrC behaviors in NRBs. NRB influent and effluent was collected from eleven full-scale systems, processed by solid phase extraction, and analyzed by liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. Resultant high resolution mass spectrometry data was then subject to extensive TOrC screening and statistical analysis to depict contaminant behavior patterns in NRBs. Fold change analyses of influent and effluent in parallel to heat map visualizations facilitate prioritization of spectral features of interest. Fold change analysis of unique spectral features groups features together that are likely removed or potentially formed TOrCs. Heat map visualizations highlight distinct feature clusters that are subsequently screened against curated suspect lists of known wastewater TOrCs. More than one hundred TOrCs have been tentatively identified and 36 have been confirmed with reference standards. Within the confirmed TOrC identifications lies evidence of well-removed (i.e. caffeine), recalcitrant (i.e. sucralose), and formed (i.e. testosterone by deconjugation) TOrCs by NRB treatment. This study aims to characterize residential wastewater and describe the treatment capacity of TOrCs by onsite wastewater treatment systems. Future work aims to elucidate endpoint fate of well-removed TOrCs and inform system optimization.

**4.07.T-06 Evaluating the Effects of Antibiotics on the Biological Transformation of Nitrogen and Pharmaceutical and Personal Care Products Removal from Onsite Wastewater in Nitrifying Sand Columns**

***Patricia Clyde***[1], *Rachel Smolinski*[2], *Roy Price*[2], *Arjun Venkatesan*[2] *and Bruce Brownawell*[2], *(1) Gradient, (2) Stony Brook University*

One reason antibiotics in the environment are concerning is their toxic effect on microbial communities. Healthy microbial communities are essential for effective biological treatment of wastewater as employed in conventional and onsite wastewater treatment systems (OWTSs). Since OWTSs don't have the benefit of dilution from a large sewershed like municipal plants do, a single resident's use of antibiotics can result in wastewater concentrations in OWTSs that are magnitudes higher than those typically observed in municipal plants. However, the effects of high concentrations of antibiotics periodically occurring in sewage on the microbial communities responsible for sewage treatment are not well understood. This study evaluates the effects of two commonly prescribed antibiotics – sulfamethoxazole and ciprofloxacin – on the removal of nitrogen and 23 pharmaceuticals and personal care products (PPCPs) from wastewater by column-scale nitrifying sand filters. The objectives of this study are (i) to observe the effect of a realistic dose of antibiotics on the nitrification process and on PPCP removal in the nitrifying filter, and (ii) to evaluate the recovery of the nitrification and PPCP removal processes if an effect due to antibiotic dosing is measured. Antibiotics were continuously added to the column influent for a ten-day period – similar to an individual's standard course of treatment – at concentrations of 150 and 750 µg/L sulfamethoxazole and 300 µg/L ciprofloxacin. Nitrogen data indicated that all three antibiotic treatments temporarily inhibited nitrification of ammonium to nitrate compared to control columns. However, recovery of the nitrification process occurred immediately after column exposure to the antibiotics was ended. No effect of either antibiotic on PPCP removal was observed. This is the first study to use environmentally relevant doses to assess antibiotic effects on microbial function in column-scale treatment systems. The results show that at concentrations relevant to OWTSs, antibiotics can affect nitrogen removal in unsaturated nitrifying sand filters, but the results also demonstrate the resilience of the treatment system.

**4.07.T-07 Evaluating Paraben Transformation Product Release in Wastewater Treatment and Changes in Paraben and Paraben Transformation Product Concentrations at Sites Downstream of Wastewater Treatment**

***Michael Penrose*** *and George P. Cobb III, Baylor University*

Disinfection processes are utilized in wastewater treatment with the intent to eliminate microorganisms and improve water quality. However, these treatments also interact with chemicals entering the wastewater system, resulting in unexpected disinfection byproducts. This study quantifies parabens, and their transformation products in wastewater treatment plants. Parabens are well removed in wastewater treatment via transformation resulting in paraben transformation products. The goal of this research is to identify and quantify transformation products released after wastewater treatment and evaluate differences in concentrations upstream and downstream of treatment facilities. Five parent parabens and nine transformation products were quantified in influent and effluent, two wastewater treatment sites at both wastewater treatment products to evaluate transformations during treatment as well as release into surface water. The five parent parabens included were methyl paraben, ethyl paraben, propyl paraben and butyl paraben. Major degradation products such as para-hydroxybenzoic acid (PHBA) and 3,4-dihydroxybenzoic acid (DHBA) were detected in influent. Chlorinated and hydroxylated parabens were also detected in influent and effluent. The same compounds were evaluated in the Brazos River upstream and downstream of treatment. Compounds were quantified using UPLC-MS. Methyl paraben was quantified in greater concentrations than any of the other parent parabens with an average yearly concentration of $2.52 \times 10^{-2}$ nM in influent and $4.54 \times 10^{-3}$ nM in effluent. The average methyl paraben concentration at the closest upstream site was $9.81 \times 10^{-3}$ nM and $4.94 \times 10^{-3}$ nM at the downstream site. Parent compound concentrations increase downstream, which means parabens are likely entering the environment from other sources. A downstream tributary has been evaluated and has quantifiable concentrations of parent parabens, indicating the tributary as a possible source of the increase in paraben concentrations. Dichlorinated methyl paraben was quantified in higher concentrations in effluent at $6.58x10^{-3}$ nM while the average concentration in influent was $3.07x10^{-3}$ nM. The mixing of effluent with river water resulted in noticeable increases in dichlorinated paraben concentrations with the highest concentration being at the post treatment site. Only minimal decreases in dichlorinated parabens were seen further downstream. Chlorinated parabens were not detected in the tributary.

**4.07.T-08 Evaluation of Innovative/Alternative Septic Systems for the Removal of Contaminants of Emerging Concern**

***Susan T. Glassmeyer***[1]*, George Heufelder*[2]*, Brian Baumgaertel*[2]*, Paul M. Bradley*[3]*, Maura Donohue*[1]*, Laura Erban*[1]*, Edward Furlong*[3]*, Timothy Gleason*[1]*, Dawn King*[1]*, Dana Kolpin*[3]*, Rachael Lane*[3]*, Elizabeth Medlock Kakaley*[1]*, Timothy McCobb*[3]*, Marc Mills*[1]*, Mallikarjuna Nadagouda*[1]*, Sheree Pagsuyoin*[4]*, Jorge Santo Domingo*[1]*, Kelly L. Smalling*[3]*, Daniel Tettenhorst*[1] *and Daniel Tush*[3]*, (1) U.S. Environmental Protection Agency, (2) Massachusetts Alternative Septic System Test Center, (3) U.S. Geological Survey, (4) University of Massachusetts, Lowell*

Groundwater is susceptible to elevated nitrogen concentration and co-pollutants from a variety of sources, including onsite wastewater treatment systems. For example, conventional onsite septic systems can release high levels of both nutrients (e.g., nitrogen and phosphorus) and contaminants of emerging concern (CECs) to groundwater and, in turn, into nearby receiving surface waters. In some areas, centralized wastewater treatment is not practically possible due to cost, lack of political will, or physical limitations; as such, septic systems should no longer be considered a temporary measure in advance of sewers but rather a permanent part of residential wastewater treatment infrastructure. Some coastal communities have been evaluating the removal of conventional septic systems and the installation of innovative/alternative (I/A) septic systems to reduce nutrient loads. Communities can select from various proprietary and non-proprietary I/A systems, each with its specific benefits and limitations. Enhanced I/A systems that include a lignocellulosic carbon source have been demonstrated to efficiently remove nitrogen from effluent along with some CECs. The removal of CECs during I/A treatment may be a co-benefit of installing these systems for residential wastewater treatment. Conversely, if some critical CECs are not removed (or only minimally removed) by the I/A treatment, communities may choose to opt for sewers instead of installing I/A septic systems.

This project examines the removal from effluent of over 280 chemical and microbial CECs by conventional and I/A septic systems. Two conventional treatments were sampled: 1) a standard pipe-in-stone trench with 1.3 m of vadose zone and 2) a drip disposal system where the septic tank wastewater disposal is in the active bio-zone (root zone) underneath the turf with percolate collected at a 0.6 m depth. Effluent was collected from 4 points in 3 different I/A systems: 1) a saturated wood layer system; 2) a denitrifying system with an aeration chamber and denitrification chamber; and a second denitrifying system with collection points after both the 3) nitrifying bed and 4) wood chip bioreactor. This study will fill a critical information gap about the impact and/or mitigatory effect I/A systems have on ground and surface water quality as a diffuse source of CEC pollution. In addition, it will provide important contributions to the growing body of literature on the performance of I/A septic systems.

**4.07.P Chemicals in Domestic and Industrial Wastewaters: Occurrence, Fate, and Use as Tracers**

**4.07.P-Tu097 Chemical Substances in Canadian Municipal Wastewater - Get your Data Here!**

***Sarah Gewurtz****, Alexandra Auyeung, Steven Teslic and Shirley Anne Smyth, Environment and Climate Change Canada*

The Government of Canada launched the Chemicals Management Plan (CMP) in 2006, which aims to reduce the risk posed by chemical substances to human health and the environment. In 2009, the CMP was expanded to include a wastewater monitoring program led by the Wastewater Science Unit of Environment and Climate Change Canada. The purpose of this program is to generate data on the concentrations of priority substances in wastewater and biosolids from representative municipal wastewater treatment plants (WWTPs) in Canada. Since 2009, the Wastewater Monitoring Program has included over 80 WWTPs. Wastewater and biosolids data from this monitoring program provide important information regarding a major pathway for chemicals entering the environment and the effectiveness of different wastewater treatment processes. However, data produced by federal departments and agencies have not historically been easily accessible to the public. In response, the Government of Canada launched the Open Government initiative as a commitment from the federal government to be transparent, accountable, and to engage with Canadians. This includes the sharing of data through an Open Government Portal. To date, the Wastewater Monitoring Program has published nine years of data (2010-2019) on seven priority substance groups to the Portal including: hexabromocyclododecane, bisphenol A, tetrabromobisphenol A, triclosan, nonylphenols and ethoxylates, halogenated flame retardants, and organophosphate flame retardants in wastewater and biosolids. Upcoming datasets include polybrominated diphenyl ether and other brominated flame retardants, and per- and polyfluorinated alkyl substances. These data can be used in a wide variety of ways. For example, the data can be used to evaluate chemical use and trends in Canadian communities and to assess the effectiveness of government regulations. The data can also be used to assess concentrations of contaminants emitted from WWTPs to the environment via effluent and application of biosolids to agricultural fields as well as the effectiveness of removal of contaminants from WWTPs. This presentation will provide an overview of the monitoring program and a summary of the datasets that are and will be available through the Open Government Portal. It is our hope that our shared data will inspire other wastewater research and monitoring programs and spark collaborations to further develop the field of wastewater science.

**4.07.P-Tu088 Multi-Element Quantification of Nanoparticles in Sludge and Wastewater Using Single Particle ICP-MS**

***Jenny Nelson Nelson****, Agilent Technologies, Inc.*

It is well understood that it is essential to quantitatively monitor the presence of nanoparticles (NPs) (engineered, natural or incidental) to understand their potential environmental and ecotoxicological implications. A significant number of NPs may migrate through wastewater treatment plants, and be released into the environment. NPs can end up in either the treated effluent or in wastewater sludge. This presentation will share a fast and simple protocol for full quantitative multi-element analysis of metallic or metal-containing NPs in wastewater and sludge samples via single-particle ICP-MS. In wastewater samples, particle mass concentrations ranged from less than 1 ng/L for Cd-based NPs to almost 100 µg/L for Mg particles. NPs from many elements were detected in wastewater, and were under 100 nm, with some exceptions.

**4.07.P-Tu089 Are Biofilm-Based Wastewater Treatment Processes Better at Removing Organic Micropollutants?**

***Narasimman Lakshminarasimman****, Sondus Jamal, Leslie Bragg, Mark R. Servos and Wayne Parker, University of Waterloo, Canada*

Conventional wastewater treatment processes are suspended growth systems where microbes metabolize the pollutants in an aerobic environment. These processes have shown incomplete removal of organic micropollutants (OMP) resulting in potential degradation of the downstream aquatic ecosystem. The membrane aerated biofilm reactor (MABR) is an emerging technology with a biofilm mode of biomass retention and different redox zones. The biofilm in MABR supports diverse classes of microorganisms to perform organics removal, nitrification, and denitrification simultaneously. It is currently unknown if the MABR design can enhance the removal of OMPs when compared with conventional designs.

To test this research question, we are investigating the first full-scale MABR installation in Canada at the Hespeler Wastewater Treatment Plant in Ontario. Composite samples of the raw influent and secondary effluent were collected on three consecutive days during select months from 2020-2022 to establish a baseline before upgrade. The samples were processed through solid phase extraction and LC-QqQ MS to measure 32 OMPs that consisted of pharmaceuticals and their metabolites, hormones, and estrogenic compounds. Sample extractions were also done for the Yeast Estrogen Screen assay to determine the reduction in estrogenic activity by the plant. The plant upgrade was completed in May 2022 and a complementary sampling campaign and analysis are being carried out to capture the plant performance post-upgrade.

Based on the average removals during the pre-upgrade phase, the target compounds were classified into three groups. Group 1 consisted of compounds such as ibuprofen, naproxen, and acetaminophen that were well removed consistently (>75%). Group 2 consisted of compounds that were moderately removed (75-25%) such as sulfamethoxazole, trimethoprim, and gemfibrozil. In group 3 there were recalcitrant compounds (< 25%) such as carbamazepine, venlafaxine, and fluoxetine. It is hypothesized that the compounds in group 2 and 3 may show better removal with the MABR process upgrade and that estrogenic activity may also decrease post-upgrade. The MABR design is an upcoming technology with multiple ongoing installation across the world. It has largely been evaluated only for conventional pollutants removal. Our findings on OMP treatment performance will be useful to risk assessors, regulators, and conservation authorities to assess the suitability of biofilm-based technologies like MABR.

**4.07.P-Tu090 PFAS Contamination of Agricultural Fields with Histories of Biosolid Application**

***Diana Oviedo Vargas***[1], *Seetha Coleman-Kammula*[2], *Charles Powley*[2] *and Jessica Anton*[2], *(1) Stroud Water Research Center, (2) Center for PFAS Solutions*

Per and poly-fluoro alkyl substances (PFAS) are synthetic organic compounds with a high content of carbon-fluorine bonds. Their ubiquitous use combined with their high mobility and stability have led to these compounds becoming pervasive in the environment. PFAS contamination of soil and water in agricultural areas can occur through the use of biosolids from wastewater treatment facilities as soil amendments. In the state of Pennsylvania, approximately 2.2 million tons of biosolids are produced each year, and application of biosolids to farmland is a widespread practice. However, the potential for PFAS contamination of farmland via biosolid applications has not been examined in detail. The goal of our study was to characterize the occurrence of PFAS in agricultural areas and understand how they migrate from biosolids to soils and eventually to receiving surface and groundwater bodies. In 2021 and 2022 we collected biosolid, soil, well water, and surface water samples from over 10 farms in Pennsylvania with varying histories of biosolid applications. When possible, samples were also collected from fields within the same farm but without previous use of biosolids (control fields). All samples were analyzed using USEPA Draft Method 1633, which is an isotope dilution liquid chromatography tandem mass spectrometry method that determines 40 different PFAS compounds. Preliminary results indicate that at least two farm fields receiving biosolids had concentrations of concern in soils (7 to 24 ppb of perfluorooctanesulfonate (PFOS) plus perfluorooctanoic acid (PFOA)) and well water (194 to 455 ppt of PFOS+PFOA, which are well above EPA's Health Advisory Level of 70 ppt). We also found elevated concentrations in an adjacent headwater stream (360 ppt of PFOS+PFOA). In contrast, control fields did not show detectable PFAS concentrations. In addition, the patterns we observed in the quantified compounds and their concentrations in biosolids, soil, and water indicate that biosolids are the likely source of contamination. Results from our work contribute to a better understanding of the pathways for transport and transformation of PFAS in the environment and provide insights on actions needed to prevent PFAS-contaminated biosolids from reaching agricultural lands.

**4.07.P-Tu092 Isotopically Labeled Ozone in Wastewater Treatment: Assessment of Biodegradability of OPs from Oxygen Transfer Reaction of Ozone with S- and N-Containing Moieties**

***Millaray Sierra Olea***[1], *Elaine Jennings*[2], *Thorsten Reemtsma*[2], *Oliver Lechtenfeld*[2] *and Uwe Hübner*[1], *(1) Technical University of Munich, Germany, (2) Helmholtz Centre for Environmental Research – UFZ, Germany*

Chemical oxidation with ozone is an established technology for the efficient removal of trace substances in the effluent of wastewater treatment plants. Despite its wide application, it has one drawback, the formation of potentially toxic oxidation products (OPs). To date, it is not possible to study the reactivity of all trace substances to ozone, the formation of their OPs, and their biological stability and toxicity. Therefore, a novel labeling technique was developed based on the hypothesis that ozonation of compounds with $^{18}O$ enriched-ozone ($O_3$) produces labeled OPs when their formation is caused by an oxygen transfer reaction. Heavy oxygen gas ($^{18}O_2$) was used to produce $^{18}O_3$, which was subsequently used to oxidize model compounds with N- and S-functional groups of interest. To establish and validate this new approach, venlafaxine (-$R_3N$) was chosen as a model compound for the formation of an OP with a known oxygen transfer reaction (-NO). Once the labeled OPs from N- and S- moieties were produced, they were used as an isotope tracer to assess the biodegradation of the newly formed functional groups (-NO, -$NO_2$, -SO, and -$SO_2$). Biodegradation batch experiments were performed to test our hypothesis that these OPs from oxygen transfer reactions to N- and S-containing moiety are more persistent than their parent compounds. Initial experiments were conducted using previously conditioned sand as inoculum, and groundwater spiked with sulfamethoxazole (SMX) and its labeled OPs. Within 28 days the removal of SMX was 99.5 ± 2.9 %, meanwhile the removal of labeled OP 4-nitro sulfamethoxazole (NIT) was 92.5 ± 0.3 %. These results suggest that the formation of the -$NO_2$ group caused by ozonation does not enhance the removal of an aniline containing parent compound. In ongoing experiments, the labeled OPs from aliphatic amines (metoprolol and sitagliptin), sulfides (penicillin G) and thiol moieties (tiopronin, cysteine and captopril) is evaluated. The analysis of these samples will focus on the stability and persistence of the labeled functional groups and their semi-quantification based on a representative labeled ion fragment or neutral loss using LC-MS/MS analysis.

**4.07.P-Tu094 Bisphenols in Municipal Wastewater from San Francisco Bay**

***Rebecca Sutton***[1]*, Miguel Alexander Mendez*[1]*, Chunjie Xia*[2]*, Jia Liu*[3] *and Da Chen*[4]*, (1) San Francisco Estuary Institute, (2) Indiana University, Bloomington, (3) Southern Illinois University, Carbondale, (4) Jinan University, China*

Bisphenols are synthetic organic compounds with high production volumes and broad uses including the manufacture of polycarbonate plastics, epoxy resins that line food containers and water pipes, and as a coating on thermal paper, among others. The most commonly manufactured and well-studied member of this class, bisphenol A (BPA), is an endocrine disruptor and is highly toxic to aquatic life, with a predicted no effect concentration (PNEC) of less than 1 µg/L. Human health concerns have led to bans and phase-outs of some uses of BPA, which in some cases has resulted in increased use of structurally-similar substitutes that are not as well-understood with regards to both toxicity and presence in the environment. We evaluated the presence of 16 bisphenols in treated wastewater effluent from the six largest municipal wastewater treatment facilities discharging to San Francisco Bay, the largest estuary on the West Coast of the Americas, and home to over seven million people. Bisphenol A, F, and S were frequently detected. Effluent concentrations of bisphenols will be presented, and compared with concentrations observed in urban stormwater discharging to San Francisco Bay, as well as in ambient Bay surface water and sediment.

**4.07.P-Tu095 Caffeine, nicotine, and their metabolites in coastal waters of the Iberian Peninsula: Environmental risk and use in the assessment of bathing water quality**

***Pepe Rodriguez-Gil***[1,2]*, Sandra Mozas Blanco*[3]*, Andreu Rico*[4]*, Isabel López*[4]*, Salome Martinez*[3]*, Judit Kalman*[3]*, Miguel Motas*[5]*, Unax Lertxundi*[6]*, Gorka Orive*[7]*, Juan Grima*[8]*, Osvaldo Santos*[9] *and Yolanda Valcarcel Rivera*[3]*, (1) IISD Experimental Lakes Area (IISD-ELA), Canada, (2) University of Manitoba, Canada, (3) Rey Juan Carlos University, Spain, (4) IMDEA Water Institute, Spain, (5) University of Murcia, Spain, (6) Araba Psychiat Hosp, Spain, (7) University of the Basque Country, Spain, (8) Geological and Mining Institute of Spain, (9) University of Lisbon, Portugal*

Combining environmental and human health perspectives into a one health framework for risk assessment increasingly gaining acceptance. Current regulations on bathing water quality, however, often focus exclusively on microbiological endpoints ignoring chemical pollution. Caffeine, nicotine, and their metabolites are commonly found in the environment and are frequently used markers of anthropogenic contamination. Our study quantified caffeine, nicotine, and their main metabolites (paraxanthine and cotinine), in coastal waters of

Spain and Portugal with the objective of assessing their environmental risk and their correlation with standard microbiological parameters used to determine the quality of bathing waters. Water samples were collected from 16 beaches along the Lisbon and Algarve (Portugal) coast, 18 in Cádiz (Spain), and 12 in the Mar Menor coastal lagoon (Murcia, Spain). All compounds were detected in all samples, with the exception of nicotine which was found in 97% of the samples. A tiered approach was used to characterize the risk posed by the four compounds. All measured caffeine concentrations resulted in precautionary Tier 1 hazard quotients (HQ) > 1 (max HQ = 144), while all but two samples resulted in HQ > 1 for paraxanthine (max HQ = 54). In the case of Nicotine, only 36% of the samples resulted in HQ > 1 (Max HQ = 80), while none did for cotinine. The cumulative distribution of Caffeine concentrations exceeded our most reliable benchmark concentration (chronic Tier 2) in 92% of the samples. Paraxanthine, exceeded the Tier 2 benchmark concentration in 67.5 % of the samples. Nicotine exceeded this level in only 0.1 % of cases. Cotinine concentrations resulted in a 6% exceedance. Due to lack of experimental data, however a conservative, modelled benchmark (EcoTTC) was used for paraxanthine and cotinine. In contrast to the estimated likelihood of environmental impacts due to the measured concentrations of these chemical markers, the bathing water quality of all sites at the time of sampling was considered "excellent" based on microbiological parameters. No statistical correlation was observed between any of the chemical parameters and the compiled microbiological parameters. Our results highlight that current bathing water quality assessment methodologies could benefit from the incorporation of chemical parameters, such as caffeine and paraxanthine, to provide a more comprehensive, one-health perspective.

**4.07.P-Tu096 Determination of Endocrine Disrupting Chemicals Using Mosquitofish (Gambusia holbrooki) and Liquid Chromatography-Mass Spectrometry**

***Rachael Rerrie**, Carme Kawana Arne, Daniel H Paull and Nora Demers, Florida Gulf Coast University*

Endocrine disrupting chemicals are compounds that affect the normal functioning of the endocrine system in organisms. These persistent substances can be found in personal care products, pharmaceuticals, pesticides, and manufactured products such as plastics. This study aims to determine the presence of endocrine disrupting chemicals in surface waters receiving runoff from a variety of land uses and their likelihood in stimulating masculinization in female Mosquitofish (*Gambusia holbrooki).* Such uses include communities using septic tanks, golf courses and communities using reuse water for irrigation, and storm water lakes at a public university. We collected fish and water samples from local Southwest Florida communities neighboring Florida Gulf Coast University in Fort Myers Florida. *Gambusia holbrooki* were analyzed for increase in four to six ratio of anal fins as indication of masculinization.

Water samples matching the areas where masculinization occurred were analyzed for organic compounds. This was done by using a single quadrupole mass spectrometer that utilizes liquid chromatography and mass spectrometry. From this data, we were able to identify individual components in the water samples based on molecular weight, polarity, solubility and ultimately the retention time of the ion. Prior research from our group has detected compounds including benzophenone, atrazine, ethylparaben, and sucralose. We suspect these compounds will also be present in the samples analyzed in this study.

We expected a higher amount of endocrine disruption and chemicals to be found in samples collected in communities with septic tanks in comparison to those using reuse or increased exposure to surface stormwater runoff. Rather, fish from areas using reuse water were more highly masculinized than those collected from a community using septic tanks. We will present data on the masculinity of fish samples found in these areas as well as the identities of chemicals found in water samples obtained from these communities.

The long-term outcome of this project can be used to determine the areas that are polluted with endocrine disrupting chemicals in Southwest Florida including the effects endocrine disrupting compounds have on aquatic species by observing the physical changes of the mosquitofish. This work is beneficial to help educate residents about how wastewater and stormwater has negative effects on the environment beyond those caused by excess nutrients.

## 4.07.V Chemicals in Domestic and Industrial Wastewaters: Occurrence, Fate, and Use as Tracers

### 4.07.V-01 PFAS in Municipal Landfill Leachate: Occurrence, Transformation, and Sources

***Staci L. Capozzi***[1]*, Amy Leang*[2]*, Lisa A. Rodenburg*[3]*, Bharat Chandramouli*[4]*, Damon A Delistraty*[5] *and Cole H. Carter*[5]*, (1) Indiana University, Bloomington, (2) University of Washington, (3) Rutgers University, (4) SGS AXYS Analytical Services Ltd., Canada, (5) Washington State Department of Ecology*

To understand sources and processes affecting per- and polyfluoroalkyl substances (PFAS), we evaluated PFAS in landfill leachate from 17 landfills across Washington State, using positive matrix factorization (PMF). PFAS were measured in leachate in both pre- and post-total oxidizable precursor (TOP) assay samples, using an analytical method similar to EPA Draft Method 1633. Total PFAS concentrations ($\Sigma_{30}$PFAS) ranged from 61–173,000 ng/L and 580–36,000 ng/L in pre-TOP and post-TOP leachate samples, respectively, indicating that little or no precursors remained in landfill leachate. Furthermore, the TOP assay often resulted in a loss of overall PFAS mass, due to chain shortening reactions. PMF modeling of the combined pre- and post-TOP samples produced five factors that represent sources and processes. Factor 5, highly loaded with PFCAs, was dominant in post-TOP samples (explaining 52% of PFAS mass) and therefore represented oxidation of precursors. Factor 5 also explained 11% of PFAS in the pre-TOP samples, suggesting the TOP assay approximates the same oxidative process that occurs in landfills, and that chain shortening reactions (which yield biodegradable products) may also occur in landfills. We speculate main sources of PFAS in Washington landfill leachate were primarily carpet, textiles, and food packaging, based on the dominance of 5:3FTCA.

### 4.07.V-02 Assessing the Applicability of the OECD 301B Ready Biodegradability Test Method for Volatile Compounds

***Sean P. McLaughlin*** *and Roxanne Brackett, Smithers*

The OECD 301 Series of Ready Biodegradability Test Methods A through F plus the OECD 310 Headspace Method all have certain benefits and detractions for their selection as a test method to evaluate chemicals for 'ready biodegradability'. Each test method prescribes a certain test set-up which are applicable to certain chemical properties such as water solubility, adsorptivity and volatility. The OECD 301B '$CO_2$ Evolution Test' is often recommended to determine the 'ready biodegradability' of a chemical due to its direct measurement of $CO_2$ and its relatively large testing volumes along with the maximum amount of inoculum allowed within the OECD 301 Series that often makes it an ideal test. The OECD 301B test is recommended for water soluble, water insoluble and adsorbing chemicals. It is not recommended though for volatile compounds.

Although this guideline is not recommended for volatile compounds since it is a flow-through system, it does have a closed bottle prior to entering the trapping solutions and given its benefits, this presentation assesses the applicability of the OECD 301B Test Method with compounds that are volatile.

The chemical compounds acetone, methanol and hexane representing the ketone, alcohol and alkane chemical groups were chosen for this experiment, conducted according to the OECD 301B guideline while aerating at a slower rate of ~20 cc/minute. The results showed that after 28 days acetone was 'readily biodegradable' and hexane was not. Methanol also appears to be 'readily biodegradable' although the 10-day window was not well defined. This experiment demonstrates that for certain volatile compounds, the OECD 301B test method may be a viable option for 'ready biodegradability' testing.

### 4.07.V-03 Development and validation of opioid analysis in wastewater matrix for wastewater-based epidemiology

***Xiayan Ye****, Cheng-Shiuan Lee and Arjun Venkatesan, Stony Brook University*

Opioid addiction and misuse are currently a national crisis and is considered a national emergency in the US. Using wastewater-based epidemiology (WBE) can potentially offer access to near real-time data on opioid consumption. However, there are no standardized methods available for opioid quantification in wastewater samples. The goals of this study are to (i) develop and validate a standardized method to detect and quantify 26 licit/illicit drugs and their metabolites in wastewater, (ii) assess the stability of analytes in wastewater at different temperatures to determine sample storage conditions; and (iii) apply the method to wastewater samples collected from three communities. 50 ml wastewater samples were filtered using 20 μm polypropylene filter and extracted by solid-phase extraction (SPE). Data to date suggest that the SPE extraction efficiency range from 97% to 133% and recoveries of spikes range from 55.6% to 108% for 26 analytes. Ongoing work on method detection limit, stability test, and wastewater monitoring at three communities will inform on the applicability of the method for WBE approaches.

## 4.08 Environmental Forensics

### 4.08.T-01 Environmental Forensics: Lessons Learned and Approaches Toward Robust Data Analysis

***Ying Wang***, *Edward Garvey, Juliana Atmadja and Solomon Gbondo-Tugbawa, WSP USA*

The value of forensic analysis has been extensively demonstrated on multiple Superfund sites, and often provides the primary means to apportion responsibility. A robust data analysis requires (1) representative samples, (2) reliable chemical data, and (3) correct application of multivariate statistical methods. However, these important requirements have often been overlooked, leading to incorrect conclusions regarding source contributions and impacts to the environment. We have had the opportunity to examine multiple sites contaminated with numerous contaminants such as polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyl (PCB), polychlorinated dibenzo-p-dioxins and furans. This presentation will show the possible pitfalls when attempting to use multivariate forensics methods without a rigorous assessment of the dataset and a thorough understanding of the multivariate statistical methods. Specifically, we will present several examples to understand the impact of the following common errors in forensic evaluations:

1) *combining multiple datasets without proper evaluation of the differences between analytical methods and laboratory analysis*; in this example, an inter-lab comparison on parent and alkylated PAH data will be performed to show the differences between the isotope dilution method and traditional internal standard quantitation method. Principal component analysis will be used to illustrate the possibility of a false outcomes by mechanically combining datasets from different analytical methods without considering analytical differences.

2) *incorrectly applying end-member mixing models based on contaminant ratios*; in this example, several end-member mixing models based on contaminant ratios will be presented to demonstrate how to correctly apply them in estimating contributions of solids and contaminants in a two-source mixing system.

3) *ignoring the constant sum constraint (i.e., no variable is free to vary independent of all the others) intrinsic to the geochemical data;* in this example, a log-ratio approach will be discussed for the multivariate analysis of geochemical data to address the constant sum constraint.

Each example will illustrate the errors that may ensue when the analyst fails to rigorously evaluate the forensic information. Recommendations will be given with the purpose of deriving a scientifically robust analysis in forensic evaluations.

### 4.08.T-02 A Weight of Evidence Approach to Filling Data Gaps in Forensic Analyses for Contaminant Source Identification

***Erin Warlow***, *Nicholas Rose and Michael J. Bock, TIG Environmental*

Forensic scientists are frequently called to conduct analyses on pre-existing data collected for purposes not directly related to the forensic investigation such as data designed to support the remedial investigation, risk assessment, or remedial design. This often means that practitioners must use a dataset limited in scope of samples, analytes, and/or geographic coverage. In many such cases, it is not practical or feasible to collect additional high-resolution data for forensic analysis. This is especially true at legacy or complex multi-party sites. While imperfect datasets present roadblocks in performing a forensic analysis for source identification, a holistic weight of evidence approach can complement the forensic analysis and allow the scientist to draw meaningful conclusions.

When a forensic evaluation is done in a vacuum without an understanding of the site history or the conceptual site model, gaps in the analytical record can stall the analysis or lead to erroneous conclusions. In our experience, some analysts may dismiss useful (but imperfect) data without reaching any meaningful conclusions, while others may speculate on potential sources that would be readily rejected based on a review of site history. To avoid these extremes, we recommend that practitioners employ other available lines of evidence to support reaching coherent conclusions in line with a well-reasoned conceptual site model. Techniques that forensics practitioners may overlook include: an understanding of known or likely historical contaminant discharge pathways, marker contaminants for a particular operation or process, data from other site media, or data and findings from comparable sites in the academic literature. In this presentation we will present common pitfalls related to use of imperfect datasets in forensic analysis without regard for other available evidence. We will then offer examples of solutions using a holistic weight of evidence approach for source identification and how these solutions can complement an imperfect analytical dataset.

**4.08.T-03 Sources of Polychlorinated Dibenzo-p-Dioxins and -Furans (PCDD/Fs) to the New York/New Jersey Harbor**

*Mahdi Chitsaz, Kelly L. Francisco and **Lisa A. Rodenburg**, Rutgers University*

The New York/New Jersey Harbor is impacted by many sources of polychlorinated dibenzo-p-dioxins and -furans (PCDD/Fs), including a large historic source of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the Passaic River. Sediment sampling conducted in 2019 as part of the Contamination Assessment and Reduction Project revealed that in some areas of the Harbor, the largest single contributor to dioxin-like toxicity in sediment is no longer TCDD, but rather is now 2,3,7,8-tetrachlorodibenzofuran (TCDF). Forensic examination of PCDD/F and polychlorinated biphenyl (PCB) fingerprints in sediments from locations throughout the harbor suggest that the main source of TCDF may be combustion of biogas, including flaring of landfill gas. A loadings assessment suggests that this is one of the few processes that produce PCDD/Fs for which emissions have not declined in recent decades. In contrast, remedial measures at the Passaic River release site, decreased use of fossil fuels, and other measures have resulted in decreasing loads of most other PCDD/F congeners.

**4.08.T-04 Atmospheric Measurements of Volatile Methyl Siloxanes in New York City**

***Christopher Brunet**, Keri Hornbuckle and Rachel Marek, University of Iowa*

Volatile methyl siloxanes (VMS) are a class of chemical compounds ubiquitous in personal care products such as deodorant, lotion, and hairspray. Because of their high vapor pressures, the majority of the volatile methyl siloxanes contained in these products are released into the air when consumers use them. Due to these large emissions as well as the fact that VMS have long atmospheric lifetimes and no natural sources, VMS have been suggested as chemical markers of urban airmasses. However, before they can be used for this purpose, the scientific community must gain a better understanding of the spatial and temporal variation of VMS concentrations near major cities. To address this need, we conducted a one-month field campaign in New York City which is expected to be one of the largest sources of VMS in the world due to its high population density. During this study, regular measurements of atmospheric VMS concentrations, meteorological conditions, and the concentrations of other relevant chemical species were collected. In this presentation, we report the early results from this study with a particular focus on how the measured concentrations compare to previous measurements from other large cities and how VMS concentrations vary diurnally in New York City. In addition, we will share our initial hypotheses about how factors such as meteorological conditions, population density, and the timing of VMS emissions may explain these results.

**4.08.T-05 Reconstructing Trends of Per- and Polyfluoroalkyl Substances (PFAS) Release and Deposition from Sediment Cores from an Urban Estuary**

*Michaela Cashman[1], Maya Morales-McDevitt[2], Anna Robuck[3], Mark Strynar[1], James McCord[1] and* ***Mark Cantwell****[1], (1) U.S. Environmental Protection Agency, (2) University of Rhode Island, (3) Icahn School of Medicine at Mount Sinai*

Per- and polyfluoroalkyl substances (PFAS) have been used extensively in commercial and industrial applications for decades. This has resulted in their widespread presence in aquatic ecosystems and raised concerns regarding their potential to impart adverse ecological effects. In this study, a series of sediment cores were collected from a site in the Delaware River Estuary, NJ, where PFAS were used extensively in manufacturing processes. The sediment cores were analyzed to characterize the source and temporal distribution of PFAS in this area of the estuary. Targeted analysis of 24 legacy PFAS showed longer chain compounds >C10 predominating in all cores despite documented local, high-volume usage of PFNA (C9). A class of recently identified chlorinated polyether carboxylic acids (Cl-PFPECAs) measured by targeted analysis were present in all cores indicating their sorption and partitioning to sediments. Downcore trends in several cores showed a clear shift in composition between the legacy and Cl-PFPECA's indicating a transition to these novel PFAS for manufacturing. Other PFAS were present in the cores at varying concentrations reflecting their discharge and accumulation in sediments over time. Data from the cores demonstrate that numerous PFAS have been released to the waters of the estuary providing evidence of long-term preservation, particularly for higher molecular weight compounds. Non-target analysis is underway to identify other PFAS present in the sediments in this area.

**4.08.T-06 Assessment of Polychlorinated Biphenyl Emissions from the Portland Harbor Superfund Site into the Atmosphere**

***Alexis Slade****, Andres Martinez, Brandi Janssen, Jessica Andino and Keri Hornbuckle, University of Iowa*

The Portland Harbor Community Coalition (PHCC) is an organization concerned about the Portland Harbor Superfund Site. The Portland harbor is contaminated with many hazardous substances, including polychlorinated biphenyls (PCBs), and the community asked us to evaluate the potential for PCB emissions from the site to their community because of dredging remediation. The Portland Harbor has historical and cultural resource significance making it one of the most complex Superfund sites designated by the Unites States Environmental Protection Agency (EPA) because of their long history of shipping, industrial and commercial activity, and its key location on the Willamette River. We hypothesized there were elevated PCB concentrations in the atmosphere surrounding the Portland Harbor due to emissions and dispersion of PCBs from the contaminated water and that these emissions from the harbor contributes to inhalation risks to the surrounding community. To address this hypothesis, we conducted a study evaluating PCB emissions from the harbor, predicted dispersion of those emissions using EPAs regulatory model system AERMOD, and conducted measurements of airborne PCBs in the community to evaluate the predictive model. Working with the PHCC we identified monitoring sites along the harbor where we requested hosts who live in the area to allow placement of samplers on their properties. We used polyurethane foam passive air samplers (PUF-PAS), which were deployed for a 6-week period, to capture and measure long term airborne PCBs. Samples were extracted and then analyzed using Tandem Mass Spectrometry GC-MS/MS (Agilent 7000) in multiple reaction monitoring (MRM) mode to quantify PCB congeners. PCB water concentrations were obtained from EPA water quality monitoring reports from 2018 to 2019 and we determined emissions using PCB concentrations and associated metadata (water velocities and sampling locations) collected and provided by the EPA. Our overall goal was to improve our collective understanding of the ambient air concentrations of PCBs and respond to and engage with the potentially impacted community of Portland Oregon.

**4.08.T-07 Source apportionment of Polychlorinated biphenyls (PCBs) in sediment using different receptor models: A case study on Portland Harbor Superfund Site, Oregon, USA**

***Mike Dereviankin*** *and Courtney Sandau, Chemistry Matters Inc., Canada*

Techniques in multivariate statistics are used by a wide variety of investigations in environmental forensics. Many of these investigations only put emphasis on the outcome of a single multivariate statistic that the expert has familiarity with using. However, it is surprisingly rare for studies to show a detailed understanding of the uncertainties created by multiple multivariate statistical techniques or how uncertainties in chemical analysis impact statistical modelling outputs. These models are most often untrained, and the forensic investigation does not involve a "data exploration", step. The outcome of different statistical models may vary and create contention between experts. The fact that a single model can provide different results is rarely acknowledged. In this presentation, these discrepancies between statistical models are investigated through the analysis of four different receptor models (NMF, ALS, PMF & PVA) used to perform source apportionment of polychlorinated biphenyls (PCBs) in sediments from Portland Harbor. The results showed that models generally had a strong agreement and identified the same main sources of PCBs. However, subtle differences were identified by; 1. different models, 2. the same models but with a different number of end-members, and; 3. the same model with the same number of end members. As expected, identifying different sources results in the relative proportion of these sources varying substantially. Depending on which method is selected, it may have a large impact on the conclusions of a scientific report or litigation case and ultimately, allocation on who is responsible for paying for remediation. Therefore, care must be taken to understand these uncertainties to select a method that produces consistent results with end members that can be chemically explained. Steps on determining the most scientifically defensible or most correct outcome will be discussed along with how to rationalize the concluding end members and model outputs

**4.08.T-08 Is PCA for statistical fingerprinting still relevant in the age of machine learning?**

***Michael J. Bock*** *and Nicholas Rose, TIG Environmental*

In the age of big data, machine learning (ML) methods such as t-SNE and UMAP have been developed to analyze and classify high dimensional data. Increasingly these methods are being used for statistical fingerprinting in environmental forensics. T-SNE and UMAP are closely related methods that use non-linear dimensional reduction to maximize the variability captured. This contrasts with PCA which uses linear regression to maximize the variability captured in the lowest numbered principal components. Plots of the first two or three principal components are retained for analysis, although the higher-numbered components are still available and can be analyzed. We explored the trade-off associated with the preservation of more of the data structure using the non-linear ML methods (t-SNE and UMAP) relative to PCA, the stochastic vs deterministic nature of these methods, and the ramifications of non-linear vs linear methods. We applied these methods to datasets that represent different environmental sources and typical environmental alteration processes that can influence chemical profiles including (1) simple mixing of sources (2) physical weathering processes such as chromatographic separation or differential solubilities, and (3) transformation processes such as the conversion of PFAS precursors to PFAS compounds. The analyses demonstrate some critical differences in the understanding of sources and processes associated with these different methods. We found that the ML methods are able to differentiate a larger number of unique sources. When many sources are present, PCA can be easily overwhelmed by the most extreme sources, masking more subtle differences. However, the ML methods were found to often be ineffective in depicting mixing between sources in a meaningful way. In contrast, mixed sources typically manifest as a straight-line connecting the sources in PCA space. The ML methods were also found to be ineffective in meaningfully depicting weathering processes and transformation processes. In contrast, these processes typically manifest as a curved trajectory in PCA space. These results show that while these new methods provide powerful tools, they often fail to provide meaningful insight into important processes such as source mixing and transformation processes. PCA continues to be a powerful tool for understanding processes that are critical to a forensics analysis.

## 4.08.P Environmental Forensics

### 4.08.P-We114 An assessment of Per- and polyfluoroalkyl substances (PFAS)-contaminated sediment transport in an urban river

***Michaela Cashman***[1]*, Maya Morales-McDevitt*[2]*, Anna Robuck*[3]*, Laura Coiro*[1]*, Thomas Boving*[2] *and Mark Cantwell*[1]*, (1) U.S. Environmental Protection Agency, (2) University of Rhode Island, (3) Icahn School of Medicine at Mount Sinai*

The continuous use and discharge Per- and polyfluoroalkyl substances (PFAS) since the 1950s has resulted in their ubiquitous distribution in aquatic matrices. While PFAS transport mechanisms are well studied in aqueous phase surface waters, little is known about their fate in solid phase or sediment transport. This study focuses on the transport of PFAS via suspended solids (SS) downstream of a site with known PFAS sediment contamination. A sediment trap was deployed in the Pawtuxet River (Rhode Island, USA), 0.5 km upstream of the river mouth to Narragansett Bay and swapped out monthly for the duration of a 12-month study. The selected site is downstream of several small wastewater treatment facilities, and 5 km downstream of a known site with PFAS contaminated sediments. Weekly water samples were taken alongside the sediment trap to assess PFAS concentrations in the water column. These results were normalized to stream height and volume using a proximal USGS stream gauge to approximate PFAS mass loading via aqueous and SS over the course of one year as part of a time-series analysis. Concentrations of ∑PFAS in the water column ranged from 10-91 ng/L over the course of the year (x=31 ng/L) and showed high variability that were not correlated to changes in flow or season. The ∑PFAS concentrations in SS ranged from 6-24 ng/g (x=15 ng/g). Strong correlations were observed between sediment concentration and SS mass in linear regressions for many PFAA compounds, including PFDoA ($R^2$=0.94), PFOA ($R^2$=0.81), and PFOS ($R^2$=0.93). The correlations between increased PFAA concentrations with increasing SS are evidence of contaminated sediments transporting downstream and contributing to overall PFAS through SS. These results signify the importance of sediment mobilization and transportation when considering PFAS source characterization for riverine environments.

### 4.08.P-We115 A Site-Specific Chemometric Approach for Assessing Per- and Polyfluoroalkyl Substance Signatures

***Skyler Sorsby****, WSP*

Ubiquitous sources and complex environmental fate properties make per- and polyfluoroalkyl substances (PFAS) difficult to assess on contaminated sites. The synchronous emergence of PFAS and the democratization of machine-learning tools directs much attention to big-data approaches that “learn” the patterns of various source types, like aqueous film-forming foams (AFFF), from many reference samples and a wide range of sites and apply them to classify site-specific samples from unknown origins. However, variable PFAS transport properties and spatiotemporal precursor alteration imprint potentially confounding patterns that can hinder supervised machine-learning (e.g., classification) algorithms. Further, the relative dearth of established source fingerprints (AFFF and otherwise) heightens the potential for incomplete or incorrect classification. Addressing this pitfall would require re-fitting supervised machine-learning models as new source profiles are published, which creates the additional risk of inconsistent results over time that may alter the conceptual site model after important milestones have been reached (e.g., remedy selection or closure approval).

This work revisits a classic PFAS case study (Ellsworth Air Force Base) and uses traditional chemometric algorithms (clustering and alternating least squares) and multiblock methods to quantify varying fingerprints in the vadose and saturated zones and potentially enhance understanding of precursor behavior. A “small data” approach such as this has the possible dual benefit of elucidating site-specific subsurface transport patterns and isolating contributions from potential sources. Archival of site-specific alteration and source fingerprints could provide a framework for later multi-site signature evaluations, and enable comparison with new PFAS reference profiles as they are discovered, avoiding the risk associated with re-fitting supervised machine-learning models.

## 4.08.V Environmental Forensics

### 4.08.V-01 Source attribution of PFASs in human serum for a paper-mill impacted community

***Ying Guo***[1], *Rachel Bauer*[1], *Ankita Bhattacharya*[1], *Sarah Choyke*[2], *Christopher Higgins*[3], *Heather M. Stapleton*[4], *John Adgate*[5] *and Courtney Carignan*[1], *(1) Michigan State University, (2) Eurofins Environment Testing (EET), (3) Colorado School of Mines, (4) Duke University, (5) Colorado School of Public Health*

Accumulating evidence has linked multiple adverse health effects with poly- and perfluoroalkyl substances (PFASs), which are being discovered in drinking water across the globe. However, accurately apportioning contamination sources remains a major challenge. Therefore, we are investigating source attribution for PFASs in serum for a Midwestern community with historic (pre-2018) PFAS drinking water contamination (1600 ppt). We enrolled 129 participants from 92 homes, administered dietary surveys, collected samples of serum and drinking water, and collected data on historic drinking water concentrations. We investigated 48 PFASs in water from private wells and found elevated concentrations of nine more not previously identified. Serum concentrations were elevated compared to the general population for PFOS, PFOA, PFHxS, PFHpS and NMeFOSAA (median=4.4, max=160 ppb). We will assess the effectiveness of three source apportionment methods (principal component analysis-multiple linear regression, positive matrix factorization, and UNMIX) to identify contributors to the serum PFAS concentrations. We expect the predominant mixture will include the PFASs elevated in the historic drinking water.

## 4.09.P Environmental Understanding: What's New with Nanomaterial and Nanocomposites in the Environment

### 4.09.P-Tu099 Characterization of Incidental Inorganic Nanoparticles within Aerosol Emissions from 3D Printing via Field Flow Fractionation and Inductively Coupled Plasma-Mass Spectrometry

*Logan N Rand*[1], *Derek Peloquin*[2], *Eric Baumann*[1] *and* ***Todd Luxton***[1], *(1) U.S. Environmental Protection Agency, (2) U.S. Food and Drug Administration*

While engineered nanomaterials have been recognized as an emerging contaminant of concern for over a decade, in recent years the release of incidental nanoparticles as unintended byproducts of human activity have also gained attention for environmental risk assessment. Desktop 3D printing has been found to emit ultrafine, often nanometer, aerosol particles which may be categorized as incidental release and pose an inhalation health hazard. Prior studies of 3D printing particle emissions have focused on aerodynamic size and concentration using airborne particle spectrometry as well as electron microscopy. Chemical analyses have indicated the production of volatile organic carbon species from polymer breakdown as well. However, the plastic feedstock is also expected to contain metal additives, which pose additional exposure concerns but are analytically challenging to study. In this work, we have developed a novel method of capturing and characterizing airborne 3D printing nanoparticles to determine inorganic composition in relation to particle size and concentration. A swirling vortex impinger is used to sample airborne nanoparticles directly into a liquid matrix, which is subsequently analyzed by asymmetric flow field flow fractionation (AF4) coupled with multi-angled light scattering (MALS), and inductively coupled plasma-mass spectrometry (ICP-MS). Additionally, single particle ICP-MS analysis was carried out to determine element specific particle number concentrations and size distributions. This new approach to the characterization of airborne incidental nanoparticles may be useful in other incidental release studies, such as urban and indoor air quality and exposure scenarios.

### 4.09.P-Tu100 Optimization of a Detection Method for a Novel Engineered Nanomaterial, MXenes, in Environmental Samples Using Single Particle Inductively Coupled Plasma-Mass Spectrometry

***Tracy Musgrove***, *Evan Gray and Jaclyn Canas-Carrell, Texas Tech University*

MXenes are two-dimensional (2D) engineered nanomaterials (ENMs) that possess characteristics allowing them to be used for a multitude of applications. Their versatility and applicability will cause an increase in production and use resulting in ubiquitous amounts of MXenes found in the environment. Thus, this increased use of

ENMs necessitates the need to better understand the environmental fate and effects of these MXenes. MXenes can degrade into multiple nanoparticles, and nanoparticles have recently been shown to exhibit nanotoxicity separate from its dissolution toxicity. Previous MXene research focused on first generation MXenes and not the more complex second-generation metal oxides sheet titanium carbide ($Ti_3C_2$) MXenes. Single Particle Inductively Coupled Plasma - Mass Spectrometry (spICP-MS) is considered the most accurate and sensitive method to measure mass distributions of ENMs in environmental systems and can identify ENMs from dissolved background signals at ng/L levels making it the perfect choice for evaluating MXenes in environmental samples. This proposed study will a method for the extraction, identification, and quantification of the second-generation complex metal oxide $Ti_3C_2$ MXene from environmental samples. In addition, -ion control studies will be performed to identify as well as which species are most likely to be affected.

**4.09.P-Tu101 Scientific basis for adapting technical guidelines for nanomaterials testing: the case of the OECD Test Guidelines with algae, daphnia and fish.**

***Susana Loureiro**[1], Fábio Campos[1], Nicolas Manier[2], Pascal Pandard[2], José M. Navas[3], Gerardo Pulido-Reyes[3] and Maria Luisa Fernandez-Cruz[3], (1) University of Aveiro, Portugal, (2) Institut National de l'Environnement Industriel et des Risques (INERIS), France, (3) INIA - National Institute for Agricultural and Food Research and Technology, Spain*

Some issues have been highlighted during the last years on how to apply the most commonly used aquatic ecotoxicity tests to nanomaterials (NMs), which are required in REACH and CLP regulations (Test Guideline (TG) nº 201 Algal growth inhibition test, TG 202 Daphnia sp., acute immobilization test and TG 203 Fish, acute toxicity test). Aiming at filling in some of these gaps, appropriate approaches on experimental set up and practical methods will enlarge the scientific basis for the eco-toxicological testing of NMs and generate technical recommendations. This scientific evidence will support the development of guidance which will be included as annexes to the "Guidance Document on aquatic and sediment toxicological testing of nanomaterials" (OECD Guidance Document N°317).

Initially, identification and collation of data, protocols and SOPs from completed and ongoing national and international initiatives were performed, to define possible improvements in the protocols of the aforementioned TGs allowing their application to NMs, in close collaboration with a dedicated working group established at OECD. Several NMs obtained from the JRC NM reference list have been selected as representative NMs : $TiO_2$ (NM 101,104), ZnO (NM 110, 111), $SiO_2$ (NM200), MWC nanotubes (NM400, 401), $BaSO_4$ (NM220) and Bentonite (NM600).

Protocols are now under optimization, and results of the gap analysis and of initial experiments will be presented aiming at the best approaches to improve the performance of the three assays with algae, daphnia and fish. These results include improved and appropriate methodologies regarding endpoints, measurements, test design, experimental media and dilutions.

The dispersion protocol used for some NM, showed consistency between the three labs involved within the TGs 201, 202 and 203. An enhanced protocol using organic matter is being carried out, with further developments aiming at improving NM dispersion. Algae counting methods, exposure vials, setup and mechanical effects are some of the explored methodologies and approaches tested.

NanoHarmony - This work has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 88593. This poster is part of the series on TG developments.

**4.10.P Fate, Effects, Mitigation, and Monitoring of Emerging Oilfield Contaminants in Aquatic Environment**

### 4.10.P-We116 Characterizing the Effects of Chronic Conventional Heavy Crude Oil Exposure on the Growth and Development of Larval Wood Frog (Lithobates sylvaticus)

***Adam Scott***[1]*, Aidan Guttormson*[1]*, Vince Palace*[2]*, Lisa Peters*[1]*, Gregg Tomy*[1]*, Bradley Park*[1]*, Mark L. Hanson*[1] *and Pepe Rodriguez-Gil*[1,2]*, (1) University of Manitoba, Canada, (2) IISD Experimental Lakes Area (IISD-ELA), Canada*

Despite an identified need for additional information, few studies have examined the effects of oil spills on the early life stages of amphibians. Beginning in 2019 at the International Institute for Sustainable Development Experimental Lakes Area in northwestern Ontario, Canada, the Freshwater Oil Spill Remediation Study (FOReSt) used in-lake enclosures to determine the effectiveness of non-invasive oil spill remediation techniques in different shoreline habitats. A second oil spill experiment, the Floating Wetland Treatments to Enhance Remediation (FloWTER) study examined the efficacy of symbiotic plant-microbe relationships for *in situ* degradation of oil-derived hydrocarbons. In 2021, the effects of *in situ* chronic exposure to conventional heavy crude oil (CHV) (FOReSt study) or a CHV water-accommodated fraction (FLoWTER study) on the growth, development, and behaviour of wood frog (*Lithobates sylvaticus)* tadpoles were characterized. Developmental and morphometric measurements were combined with the assessment of bioaccumulation to determine the effects of oil-spill-impacted waters on the tadpoles. Within the FloWTER project, behavioural assays were also conducted on a subset of tadpoles. In the FLoWTER mesocosms, there were no apparent treatment effects when examining total length, snout-vent length and tadpole wet weight. Bioaccumulation of total polycyclic aromatic hydrocarbons (TPAH) in tadpole whole bodies was also assessed and not statistically different among treatments. Qualitative histology analysis also supports these results, as there were no apparent differences when examining hepatic changes. Similar results were observed in the FOReSt study regarding apical endpoints and histology analysis; however, bioaccumulation of TPAH in the in-lake enclosures found statistically higher concentrations of TPAHs in oiled compared to non-oiled treatments. This research provides robust data on the toxicological effects of CHV on amphibians to inform risk assessment related to the over-land transportation of CHV and potential impacts of freshwater oil spills on amphibians.

### 4.10.P-We117 Examination of chemical and biological changes related to aged oil from historical shipwrecks

***Stanley (H.Y.) Poon****, Taylor Strong, Susan E. Cobanli, Gary Wohlgeschaffen, Brian Robinson, Tom L. King and Alice C. Ortmann, Fisheries and Oceans Canada, Canada*

A recent heavy marine oil spill was observed at Bligh Island, BC. The source oil was being released from the wreck of the *M/V Schiedyk* that sank in 1968. Bunker "C" type fuel oil was coming from one of the two fuel tanks, while the second fuel tank contained marine diesel oil. Oil in submerged tanks may undergo unknown weathering as it mixes with seawater, and as a result, it is important to understand its physical and chemical properties, and the natural attenuation rate of these aged products in the marine environment.

The objective of this study was to determine the fate and behaviour of aged Bunker C and marine diesel, with a focus on how seasonal changes impact 1) hydrocarbon physical and chemical properties, 2) microbial dynamics in the presence of these hydrocarbons, and 3) biodegradation rates. Microcosm studies with Bedford Basin seawater containing either Bunker C or diesel oil collected from the *Schiedyk* were set up between 6 to 18°C. The change in hydrocarbon composition was monitored through the use of GC-MS, fluorescence spectroscopy and Microtox, while microbial dynamics were determined using 16S rRNA sequencing and flow cytometry.

At a water temperature of 18°C, Bunker C microcosms showed an overall 24% reduction in toxicity after 28 days incubation. However, diesel microcosms showed a 3-fold cell density increase in day 7, followed by a 3.8-fold toxicity increase in day 14, which may suggest the diesel biodegradation by-products may contribute to the increase in toxicity observed.

Further chemical and genomic analyses will provide important insights on hydrocarbon compositional and microbial dynamic changes during the incubation period.

Although the *Schiedyk* spill has been addressed, evidence-based advice can be generated from this study regarding future emergency spill response specifically targeting historical shipwrecks that may release aged oil in the future.

**4.10.P-We118 A simple tool for screening oil samples: coupling machine learning and fluorescence image analysis**

***Jeff A. Steevens***[1]*, Dong Xu*[2]*, Biplab Poudel*[2]*, James Tipton*[2]*, Jiacheng Xie*[2]*, Rishi Patel*[3]*, Qihua Wu*[3]*, Chloe Keyes*[3]*, Camron Satterfield*[3] *and Jacob Berry*[3]*, (1) U.S. Geological Survey, (2) University of Missouri, (3) Missouri State University*

Measuring the concentration of oil in the aquatic environment is important for determining the potential exposure, risk, or injury from legacy contamination or an oil spill. Conventional analytical chemistry methods require samples to be collected in the field, shipped, and processed in the laboratory, which is rather time-consuming, laborious, and costly. For rapid field response immediately after a spill, there is a need to estimate oil concentration in near real time. To make the oil analysis portable, fast, and cost effective, we developed a plug-and-play device and a deep learning model to assess oil levels in water using fluorescent images of water samples. We prepared approximately 1300 samples of oil at different concentrations to train and test the deep learning model. The model comprises a convolutional neural network and a novel module of histogram bottleneck block with an attention mechanism to exploit the spectral features found in low-contrast images. This model predicts the oil concentration based on a fluorescent image of the sample. Samples are prepared using a hexane solvent extraction. We developed an integrated hand-held device for capturing fluorescent images. This device captures images of samples using a cuvette and holder, camera, LED light at 385 nm and saves images on a memory card. Images are transferred to the smart phone for analysis by the application. Future development and demonstration of this smart phone application will target the oil spill response community with further expansion for citizen science applications.

**4.10.P-We119 Revision of toxicokinetic framework for evaluation of time variable toxicity of hydrocarbons**

***Aaron Redman***[1]*, Thomas Parkerton*[2]*, Abraham Smith*[1] *and Cary Sutherland*[1]*, (1) ExxonMobil Biomedical Sciences, Inc., (2) EnviSci Consulting*

Standardized aquatic toxicity tests have greatly improved the scientific understanding of the hazards and resulting risks of chemicals in the environment. However, environmental exposures are typically dynamic and require the use of toxicokinetic frameworks to scale the results derived using traditional constant exposures on the order of a few days to short term transient exposures that can occur on shorter time scales particularly in the case of accidental spills. This presentation will summarize the findings from a recent application of a one box toxicokinetic modeling framework to a database of 150 individual acute toxicity tests with hydrocarbons spanning a wide range test organisms, chemical classes, and logKow. This work is based principally on the time-course of constant exposure concentration toxicity tests but also includes several studies that involved a time to death experimental design. The key model parameter that dictates the time progression of toxicity is represented as a lumped first-order rate that was shown to range from 0.3 – 8 $d^{-1}$. The observed variability in rates were then systematically evaluated for dependence on study variables such as logKow, test temperature, and organism size and class. Unexpectedly, these factors were shown to explain only a limited amount of the observed variance in this key model parameter. Novel pulsed exposure experimental toxicity data using a flow-through passive dosing test system were also developed to further test this model framework. Finally, preliminary application of this framework to whole oil datasets will be presented and the implications of this work in refining existing effect models used in oil spill effect assessments and future research needs will be discussed.

### 4.10.P-We120 Transformation and Attenuation of Bitumen-Derived Naphthenic Acid Fraction Compounds in Athabasca Oil Sands Wetlands

***Ian James Vander Meulen**[1,2], Joanne L. Parrott[1], Danna M. Schock[3], Lukas J. Mundy[1], Chukwuemeka Ajaero[2], Monique C. Simair[2,4], Bruce Pauli[1], Kerry M. Peru[1], Dena W. McMartin[2,5] and John V. Headley[1], (1) Environment and Climate Change Canada, Canada, (2) University of Saskatchewan, Canada, (3) Palustris Environmental, Canada, (4) Maven Water & Environment, Canada, (5) University of Lethbridge, Canada*

Overburden from oil sands mines, including surface materials and lean oil sands, is necessarily displaced and stockpiled to facilitate access to rich bitumen deposits. Where landscapes are affected by legacies of historical oil sands development, there is some concern about residual bitumen-attributable toxicity, some of which can be attributed to naphthenic acid fraction compounds (NAFCs). In one reclaimed area built on piled mining overburden known as Gateway Hill, an opportunistic wetland formed along a linear north-south reclamation project boundary. Samples were collected along Gateway Wetland's flow pathway, which were then extracted and analyzed for NAFCs using high-resolution Orbitrap mass spectrometry. Attenuation of NAFCs progressed sequentially along the wetland's flow pathway. Changes in NAFC characteristics observed included decreasing concentrations of NAFCs, decreasing molecular weight of NAFCs across compound classes, and increasing abundance of highly oxidized formulae. Changes in molecular characteristics of NAFCs coincided with apparent decreases in some distress markers during development of fathead minnow embryos, evidenced by a decreasing magnitude of embryonic heart rate suppression and decreased time-to-hatch. Trends observed in NAFCs (i.e., decreasing concentrations, decreasing molecular weight, and increasing abundance of $O_x$-formulae) are consistent with previous work examining the effects of treatment wetlands under controlled conditions. This work provides evidence that mature wetland ecosystems on mining-affected landscapes may be capable of offering important ecosystem services in-line with those observed in wetland treatment systems under controlled conditions.

### 4.10.P-We121 Remediation of High Salinity Soil Using Enhanced Evaporative Flux

***Jason K. Geiger**[1], Richard F. Carbonaro[2], Dominic M. Di Toro[1], Linda J. Eastcott[3] and Paul T. Imhoff[1], (1) University of Delaware, (2) Manhattan College, (3) Imperial Oil, Canada*

At oil and gas sites, incidental releases of high salinity waters may occur, potentially impacting surrounding plants and soils. Currently, remediation approaches may involve excavating and backfilling site soil, which can be an expensive and disruptive process. To limit the need for this disturbance, a crystallization modifier can be utilized to enhance the natural evaporative flux process, allowing efflorescence of salt crystals on the soil surface. Prior studies were limited to remediation of sodium chloride solutions and did not address the variability of field conditions. The current research examines the efficacy of a crystallization modifier, ferrocyanide, on remediation of saline solutions and high salinity brines in column experiments using five media (three uniform sands and two field soils) spanning a range of soil textures, organic matter content, and other soil characteristics. Experiments were conducted under conditions representative of summer and winter in humid and dry North American climates. Enhanced evaporative flux typically removed 60-90% of salt to the soil surface across all soils and environmental conditions. Results of this study can inform the environmental conditions under which enhanced evaporative flux is effective and the potential need for co-treatment (e.g., wicks, tarps) in challenging environmental settings.

### 4.10.P-We122 Simulating Enhanced Evaporative Flux Remediation of High Salinity Soils Using a Modified HYDRUS-PHREEQC-1D Model

***Richard F. Carbonaro**[1], Jason K. Geiger[2], Dominic M. Di Toro[2], Linda J. Eastcott[3] and Paul T. Imhoff[2], (1) Manhattan College, (2) University of Delaware, (3) Imperial Oil, Canada*

Incidental releases of high salinity brines during oil and gas operations may impact surrounding plants and soils. When a release occurs, evaporative flux naturally transports solutes toward the soil surface but will eventually slow when salts precipitate in soil pores. Crystallization modifiers can enhance the evaporative flux process, delaying the onset of precipitation and allowing efflorescence of salts above the soil surface. This approach may reduce the need for soil excavation and backfilling, limiting the impact of site remediation on the environment. During evaporative flux, salt precipitation often causes pore-clogging and a reduction in soil permeability. The impact of pore-clogging during transport processes has not been fully elucidated. To elucidate the process occurring in *Remediation of High Salinity Soil Using Enhanced Evaporative Flux*, the HYDRUS-PHREEQC-1D model was applied to characterize the transport and precipitation of salts and subsequent impact on hydraulic parameters. The model was calibrated using laboratory column experiments for a range of environmental conditions and soil textures. This model can be used to make predictions regarding the amount of crystallization modifier required for remediation and the duration of remediation.

## 4.10.V Fate, Effects, Mitigation, and Monitoring of Emerging Oilfield Contaminants in Aquatic Environment

### 4.10.V-01 Sedimentary eDNA metabarcoding reveals that PAHs contamination is linked to river phytoplankton and fish biodiversity loss

***Yongrong Hao*** *and Jiahua Guo, Northwest University, China*

River sediment is the habitat for benthic organisms, and the source and sink of pollutants. Benthic ecosystem, composing of bacterial, fungi, algae, protozoan, metazoan communities, and bottom-dwelling fish, is a complex biological community across multiple trophic levels. Yet few study has attempted to evaluate the potential effects of polycyclic aromatic hydrocarbons (PAHs) contamination on the biodiversity of the whole sedimentary communities. In this study, Beiluo river located in the Loess Plateau (China) was used as a representative site to reveal the river sediment community shifts and potential interaction with PAHs. We hypothesize that low molecular weight (LMW) PAHs in Beiluo River was the primary stressor for the abundance and diversity loss for benthic communities. The objectives of this study were: 1) to investigate the distribution of multitrophic communities in sediments using DNA metabarcoding, including 16S-, 18S-, COI- and 12S rRNA sequencing for bacteria, microbial eukaryotes, metazoan, and fish, respectively; 2) to reveal the spatial distribution levels of PAHs and source apportionment; 3. to unravel whether potential PAHs in watershed sediments links to the shifts in community structure and diversity. Here, the concentrations of PAHs in sediments ranged from 25.95 to 1141.35 ng/g. Furthermore, the high concentrations of MMW PAHs (ANT, PHE), mainly deriving from coal and biomass combustion, were negatively linked with the Shannon's diversity index and richness of decomposer-bacteria (*Proteobacteria*), primary consumer-protists (*Apicomplesa*) and secondary consumer-fish (*Pleuronec*). High molecular weight (HMW) was linked to the reduced diversity and richness of algae (*Chlorophyta, Chrysophyceae*) and fish (*Cichliformes*), though the occurrence level of HMW was low. Surprisingly, no evident connection on the biological community was observed from the low molecular weight (LMW) PAHs deriving from the oil spill. In conclusion, MMW and HMW PAHs derived from coal and biomass combustion and vehicle exhaust in river sediments may reduce the river sedimentary biodiversity of primary producers and secondary consumers.

## 4.11 From Legacy Pollutants to Chemicals of Emerging Concern: The Great Lakes as Case Study

### 4.11.T-01 Epigenetic and Transcriptomic Changes Resulting from Long-term Exposure to Contaminant Mixture Associated with Agricultural Land Use.

***Mary Jean See***[1]*, Weichun Huang*[1]*, Nicholas Cipoletti*[2]*, Heiko Schoenfuss*[2]*, Daniel J. Sullivan*[1] *and Adam Biales*[1]*, (1) U.S. Environmental Protection Agency, (2) St. Cloud State University*

Epigenetics, the interface between the environment and genome, can change quickly in response to environmental stressors. Epigenetic modifications may cause changes in gene expression leading to potential adverse biological outcomes. Here, we present differential DNA methylation and mRNA expression from liver tissue of *Pimphales promelas* (fathead minnows, FHM) exposed to a mixture of eight chemicals for two generations. The mixture of eight contaminants (alkyl phenols, atrazine, BPA, bromacil, DEET, estrone, metolachlor, and TBEP) was developed from a watershed survey around the Laurentian Great Lakes performed by USGS from 2010 to 2014. The mixture represents chemicals found in waters receiving inputs from agricultural land use. Adult FHM were exposed to the mixture in a flow-through system for three months at a concentration approximately the same as (medium), ten times greater than (high), and ten times lower than (low) the highest measured environmental concentration along with a solvent control. Fish were allowed to breed during this period, then sacrificed for the measurement of apical endpoints and collection of tissues. Offspring of initial exposed adults were reared to adulthood in the flow-through system and continuously exposed to the same treatments. They were allowed to breed, then sacrificed for measurement of apical endpoints and collection of tissues. DNA and RNA were isolated from liver tissue then sequenced using reduced representation bisulfite sequencing (RRBS) and global mRNA sequencing. Differential DNA methylation patterns along with gene expression results suggest a response to a weakly estrogenic treatment. Males in both exposed generations displayed an increasing trend in vitellogenin expression while females in both generations showed little or no response to the exposure. Effects of the complex mixture representing agricultural land use were weakly estrogenic and we found offspring males showed a decreasing trend in secondary sex characteristics in response weak estrogenic exposure. Our findings demonstrate epigenetic modifications occur concurrently with gene expression changes and preliminary results suggest gene expression changes are related to reproductive end points along an adverse outcome pathway, demonstrating the relevance of evaluating biological effects of environmental mixtures.

**4.11.T-02 Legacy and Emerging Contaminants in North American Herring Gull (Larus argentatus) Serum from the Laurentian Great Lakes**

***Sydney K. Brady***[1]*, Kevin A. Romanak*[1]*, Keith Grasman*[2] *and Marta Venier*[1]*, (1) Indiana University, Bloomington, (2) Calvin University*

Herring gulls (*Larus argentatus*) are a sentinel species for the Laurentian Great Lakes ecosystem. They are predators that bioaccumulate legacy pollutants through aquatic and terrestrial food sources. Therefore, concentrations of pollutants in herring gulls can be used to elucidate general trends in the concentrations and movements of legacy chemicals throughout the Laurentian Great Lakes.

Herring gull blood samples were collected between 2010 and 2021 in Michigan near Lake Huron, Lake Michigan, and Lake Erie. Target chemicals were extracted from the blood serum through liquid-liquid extraction followed by solid-phase chromatography. Flame retardants were analyzed using GC-MS EI (electron ionization) while pesticides and PCBs (Polychlorinated Biphenyls) were analyzed using GC with an ECD (electron capture) detector.

Preliminary results suggest a widespread accumulation of penta-BDE (Brominated Diphenyl Ether) congeners BDE-47, 99, and 153 in herring gull serum. Dechlorane Plus is also detected frequently, but at lower concentrations than penta-BDE congeners. All bird serum contains PCBs, of which the concentrations are predominated by congeners 118 and 180. Furthermore the pesticides and pesticide byproducts, p,p'-DDE (Dichlorodiphenyldichloroethylene), HCB (Hexachlorobenzene), Octachlorostyrene, and Dieldrin are present in numerous samples.

These data, in conjunction with existing data on legacy and emerging chemicals in herring gulls, contribute to our understanding of the spatiotemporal trends of legacy and emerging contaminants in the Laurentian Great Lakes Region.

**4.11.T-03 Temporal Trends of Legacy and Current-use Halogenated Flame Retardants in Air, Precipitation, Herring Gull Eggs and Lake Trout in Lake Ontario**

*Wen-Long Li*[1]*,* ***Hayley Hung***[1]*, Tana V. McDaniel*[1]*, Shane R. deSolla*[1]*, Lisa E. Bradley*[1]*, Alice Dove*[1]*, Daryl McGoldrick*[1] *and Paul Helm*[2]*, (1) Environment and Climate Change Canada, (2) Ontario Ministry of the Environment, Conservation and Parks, Canada*

Under Canada's Chemicals Management Plan (CMP), polybrominated diphenyl ethers (PBDEs) and alternative halogenated flame retardants (HFRs) were measured in air (2005-2017), precipitation (2006-2018), lake trout (1997-2019) and herring gull eggs (2008-2018) in the Great Lakes region. These multimedia long-term datasets were combined with a multimedia environmental model, the modified Quantitative Water Air Sediment Interaction (QWASI) model, based on site-specific environmental parameters from Lake Ontario to better understand the observed trends and environmental fate of HFRs in the region. All targeted HFRs were detected in one or more air [detection frequency (DF): 0.80-98%], precipitation (DF: 6.4-97%), lake trout (DF: 34-100%), and herring egg (DF: 8.3-100%) samples throughout the sampling periods. General decreasing trends were found for PBDEs for air, precipitation and lake trout since mid-2000, while herring gull eggs have shown little or no change in trends. Temporal trends for non-PBDE HFRs are not as clear as those for PBDEs. Hexabromocyclododecane (HBCDD) concentrations decreased from 2006 to 2011 but started to increase after 2011 both in air and precipitation. On the other hand, HBCDD showed no trends in lake trout and herring gull eggs. Model results suggest that the loadings from tributaries and wastewater effluent were the primary sources for PBDE and HFR input to the lake. Total degradation in the water and sediment were the dominant removal pathway for BDE 47, 209 and HBCDD, followed by sediment burial.

**4.11.T-04 Are you sure you want to eat that? Investigating Polychlorinated Biphenyl Concentrations in Lake Erie Fishes**

***Olivia Hodgson****, Sam Nutile, Adam Martin Simpson, Cody Wood, Jeremiah Keyes and Ashley Russell, Penn State Behrend*

Lake Erie serves as an important commercial and recreational fishery for residents of Pennsylvania, yet consumption advisories for polychlorinated biphenyls (PCBs) exist for many commonly caught and consumed game species. With consumption limits set at one meal per month due to excessive PCB contamination, there exists large potential for dietary exposure of Pennsylvania anglers to PCBs. The risk associated with consumption of Lake Erie fishes, however, is unclear as little research exists regarding the effects of PCBs on human health when dietary exposures are considered. Therefore, the objective of the current research is to document PCB contamination within filets of common game species targeted by recreational anglers within Lake Erie and model assimilation into human tissues based on dietary consumption according to fish consumption advisories. PCBs were extracted from filets of walleye, freshwater drum, yellow perch, bluegill, and steelhead and pharmacokinetic modeling was used to predict how PCBs accumulate within the tissues and organs of humans. Extractions of PCBs from filets of fish reveal concentrations of select PCBs ranging from 3.16 to 12.6 mg/kg lipid. Pharmacokinetic modeling shows that for one PCB (PCB-105), steady state concentrations approaching 600 µg of PCB would be expected to accumulate within the fat of an individual based on a consumption rate of one meal (8 ounces) of fish per month over five years. With tentative links to oncogenesis in humans, the PCB concentrations determined to be present within filets of common recreational fish species will provide a clearer understanding of the exposure risk of Pennsylvania anglers to legacy contaminants, as well as document the potential human health effects resulting for such exposure.

**4.11.T-05 Quantifying Hazards to Fish from Emerging Contaminant Exposures: A Great Lakes Basin Regional Assessment**

***Dan Gefell**[1], Amber Bellamy[1], Steph Hummel[1], Richard Kiesling[2], Sarah Elliott[2] and Aliesha Krall[2], (1) U.S. Fish and Wildlife Service, (2) U.S. Geological Survey*

Contaminants of emerging concern (CECs) have been detected extensively and consistently in fresh surface waters throughout North America. The ecotoxicological literature further indicates that many CECs are absorbed by aquatic organisms and are toxic at environmentally relevant concentrations. Yet, evaluations of regional distribution and biological breadth of hazards posed by CECs to aquatic life are limited. The U.S. Fish and Wildlife Service and U.S. Geological Survey conducted a basin-wide ecological hazard assessment in fish using an aggregate database of CEC concentrations in 7,165 surface water grab samples collected during 1991-2021 from watersheds distributed across the Great Lakes Basin in the United States and

Canada. Environmental CEC data were compiled from over 20 public sources in the peer-reviewed literature and government databases. Surface water CEC concentrations were translated into ordinal scores of relative hazard using novel CEC- and effect-specific screening values derived by the study team from published laboratory assays, resulting in a database of nearly 205,500 hazard scores. We evaluated regional spatial hazard distribution patterns and temporal patterns in select watersheds related to 16 frequently detected CECs representing a wide variety of human uses (pharmaceuticals, pesticides, flame retardants, hormones, fragrances, personal care) and 12 toxic effect categories (including mortality, reproductive, developmental, and behavioral effects, among others). Preliminary results suggest that metolachlor and DEET were associated with elevated hazard to fish more frequently than other CECs and endocrine and genotoxic effects were most often identified as potential effects. We further describe spatial associations between effect-specific relative hazard and upstream CEC point sources (wastewater treatment plants and combined sewer overflows). A basin-wide understanding of the nature and extent of CEC-related hazards to fish is important for regional fisheries management, environmental discharge regulation, and management of municipal waste streams.

**4.11.T-06 Impact of Environmental and Engineered Processes on Per- and Polyfluoroalkyl Substance Fingerprints from an Aqueous Film Forming Foam Manufacturer near Lake Michigan**

***Sarah Balgooyen*** *and Christina K. Remucal, University of Wisconsin, Madison*

Forensic analysis of per- and polyfluoroalkyl substances (PFAS) contamination sites is challenging due to the large list of analytes with varying mobility and transformation in the environment. For example, the use of fingerprinting analysis, in which the PFAS compound distribution is used to identify sources, is not always effective due to alteration of the PFAS fingerprint as a groundwater plume migrates away from the source. Additionally, engineered systems, like wastewater treatment, can further alter PFAS composition by preferential partitioning of compounds to biosolids and transformation of PFAS precursors. In this study, we investigated the change in PFAS composition due to various environmental and engineered processes, including groundwater and surface water flow, wastewater treatment, and application of contaminated biosolids. We focused on sites near Marinette, Wisconsin, which is on the shore of Green Bay of Lake Michigan and is impacted by PFAS contaminated from a fluorotelomer aqueous film forming foam (AFFF) manufacturer. We use fingerprinting analysis along with quantification of PFAS precursors to investigate potential source attribution methods following groundwater transport and application of industrially impacted biosolids. The fingerprint of terminal and intermediate PFAS (i.e., perfluorocarboxylic acids and fluorotelomer sulfonates) demonstrates that Green Bay surface water is directly impacted by the AFFF manufacturer. However, source attribution near biosolids-impacted fields is complicated by PFAS transformation and partitioning during the biosolids stabilization process and/or transport after land application.

**4.11.T-07 Per- and polyfluoroalkyl substances (PFAS) in precipitation and lake water in the Great Lakes basin**

***Chunjie Xia****, Abby G. DeMeyer, Kevin A. Romanak, Staci L. Capozzi and Marta Venier, Paul H. O'Neill, Indiana University, Bloomington*

Per- and polyfluoroalkyl substances (PFAS) have been widely used in various applications for over 70 years, leading to ubiquitous distribution in the environment. Public concern over the health risk posed by PFAS has triggered an increasing number of studies conducted to address their environmental occurrence, fate, and transformation as well as associated toxic effects. Wet deposition is an important source of PFAS transport to the environment. However, data on PFAS levels in precipitation remain limited, particularly in the Great Lakes basin. Precipitation samples were collected in 2021 from five sites in the Great Lakes region and open lake water was also collected. These samples were analyzed using a liquid chromatograph coupled to tandem mass spectrometer (LC/MS/MS) for a total of 39 targeted PFAS. Total targeted PFAS concentrations ranged from 1.53–72.8 ng/L with short-chain ($C \leq 6$) perfluoroalkyl carboxylic acids (PFCAs) being the dominant compounds in the precipitation samples. Perfluorooctanesulfonic acid (PFOS), 6:2 fluorotelomer sulfonic acid (FTS) and perfluorobutane sulfonamide (FBSA) were also widely detected in samples. The spatial difference of

PFAS concentrations between precipitation and lake water indicated that precipitation is an important source of PFAS to surface waters in the Great Lakes. PFAS levels in precipitation were also compared to levels of legacy pollutants (i.e., polybrominated diphenyl ethers [PBDEs], polychlorinated biphenyls [PCBs], and pesticides) to understand if any relationship exists between the various suites of compounds.

**4.11.T-08 Discussion - From legacy pollutants to chemicals of emerging concern: the Great Lakes as case study**

***Marta Venier**, Indiana University, Bloomington*

This discussion block is intended to allow open discussion on topics related to the session. In particular we will discuss the following questions:

- How can studies in the Great Lakes, one of the largest ecosystems in the US, aid in identifying chemicals of emerging concern?
- Has the next chemical threat already emerged? How do we identify it? What are the limits and obstacles?
- There is a large amount of information being collected throughout the Great Lakes' ecosystem. How do we ensure that this data is findable, accessible, interoperable, and reusable (F.A.I.R.)?

**4.11.P From Legacy Pollutants to Chemicals of Emerging Concern: The Great Lakes as Case Study**

**4.11.P-Tu103 Creation of a new Great Lakes fish Standard Reference Material (SRM): SRM 1947a Great Lakes Fish Tissue**

***John R. Kucklick**[1], Jessica Lynn Reiner[1], Rebecca Pugh[1], Debra Ellisor[1], Jennifer Hoguet[1], Amanda Moors[1], Jennifer Ness[1] and Brian Lantry[2], (1) National Institute of Standards and Technology (NIST), (2) U.S. Geological Survey*

The National Institute of Standards and Technology (NIST) has long provided tissue matrix reference materials value-assigned for organic contaminants to meet stakeholder needs. Two highly used fish materials include Standard Reference Material (SRM) 1946 Lake Superior Fish Tissue and SRM 1947 Lake Michigan Fish Tissue. Both materials consisted of cryogenically homogenized lake trout (*Salvelinus namaycush*) filets collected in the late 1990s. The materials were value-assigned for PCB congeners, organochlorine pesticides, polybrominated diphenyl ether congeners (PBDEs), and fatty acids. Several trace elements, including mercury isotopes, five per- and polyfluorinated alkyl substances (PFAS), and a-hexabromocyclododecane (HBCD) were also value assigned in SRM 1947. SRM 1947 is sold out and SRM 1946 will sell out within one year, therefore NIST started the process to produce a replacement fish material. In September 2021 the United States Geological Survey (USGS) collected lake trout from Lake Ontario near Oswego, New York, and provided NIST with 124 kg of lake trout filets. Lake Ontario was selected as a new collection site partly because we anticipate based on published studies that the lake trout will have higher levels of PFAS than fish from the other Laurentian Great Lakes. The material was cryogenically homogenized into a fresh, frozen powder material and once bottled, will yield approximately 1300 units (four jars x 10 g/jar) becoming SRM 1947a Great Lakes Fish Tissue. SRM 1947a will be value-assigned through NIST measurements and a multi-laboratory study for a suite of organic contaminants similar those in SRMs 1946 and 1947 although we anticipate a greater number of PFAS will be value-assigned.

**4.11.V From Legacy Pollutants to Chemicals of Emerging Concern: The Great Lakes as Case Study**

**4.11.V-01 Source Apportionment of Atmospheric Polybrominated Diphenylethers in the Great Lakes**

***Staci L. Capozzi**[1], Lisa A. Rodenburg[2], Daniel C. Lehman[1] and Marta Venier[1], (1) Indiana University, Bloomington, (2) Rutgers University*

Polybrominated diphenylethers (PBDEs) were widely used flame retardants in electronics, textiles, plastics, coatings, and polyurethane foams. Legacy commercial formulations have been voluntarily withdrawn from the market due to concerns about the effects of these compounds on the environment and public. The objective of this work was to use the database of PBDE gathered by the Integrated Atmospheric Deposition Network (IADN) to elucidate and apportion sources of PBDEs in the Great Lakes. Statistical analysis via positive matrix factorization (PMF) was applied to the dataset of PBDE concentrations in air (vapor plus particle) and precipitation. PMF was used to identify co-varying patterns of contaminants (i.e., fingerprints) that were then matched to primary and secondary sources of PBDEs in the Great Lakes. Source apportionment using PMF identified three legacy commercial PBDE mixtures in air (i.e., pent-, octa-, and deca-BDE mixtures) as well as elucidating two distinct photochemical debromination pathways. The spatial and temporal variations of individual sources within the Great Lakes were also determined. PMF has proven to be an effective tool to apportion legacy pollutants and shed light on their transformation in the atmosphere using over a decade worth of monitoring data.

**4.11.V-02 Behavioral changes in Cyprinus carpio larvae produced by environmental concentrations of aluminum, carbamazepine, ibuprofen and diazinon.**

***Misael Hernández Díaz*[1]*, Alejandra Cortes-López*[1]*, Karina Ruiz-Lara*[1]*, Selene Cano-Viveros*[1]*, Leobardo Manuel Gómez-Oliván*[2]*, Sandra Garcia-Medina*[1] *and Marcela Galar-Martinez*[1]*, (1) Instituto Politécnico Nacional, Mexico, (2) Autonomous University of the State of Mexico*

Emerging pollutants turn out to be important compounds to study since they can be found in low concentrations in natural environments. Environmental contaminants such as aluminum, diazinon, and drugs such as carbamazepine and ibuprofen have been shown to have central nervous system effects in aquatic organisms. Knowing the effects of these pollutants on the nervous system in fish larvae is important since they can modify behavioral changes. The aim of this study was to analyze the behavioral changes produced by aluminum, carbamazepine, ibuprofen and diazinon in *Cyprinus carpio* larvae. *C. carpio* larvae were placed in 24-well plates in concentrations of 50 µg/L of aluminum, carbamazepine, ibuprofen and diazinon for 12, 24, 48, 72 and 96 hours. Plates were analyzed in a DanioVision Noldus viewing chamber to assess startle response, locomotor activity, and thigmotaxis changes. It was observed that the C. carpio larvae exposed to diazinon were the ones that presented a greater increase in the distance they traveled and speed up to 72 h, they also had less immobility time compared to the control group. Larvae exposed to carbamazepine, ibuprofen and aluminum showed a decrease in their distance, as well as their speed during the 96 h of exposure, reflecting a longer time of immobility. Finally, changes in the thigmotaxis response were observed, showing that the larvae exposed to carbamazepine spent more time in the center of the well, showing periods of anxiety, unlike aluminum and ibuprofen, which spent more time in the periphery, reflecting stress stages. Aluminum, carbamazepine, ibuprofen and diazinon were shown to have effects on the behavior of *C. carpio* larvae, showing changes in their speed, distance, mobility and thigmotaxis. The results obtained in this study show that environmental concentrations of emerging contaminants can produce behavioral changes in fish larvae and this puts their health and survival at risk in aquatic bodies with low levels of contamination.

**4.12 Human Exposure to Organic Contaminants of Concern**

**4.12.T-01 Residential Exposure to Per- and Polyfluoroalkyl Substances (PFAS) via Household Dust in Eight Impacted Communities**

***Jeffrey Minucci*[1]*, Nicole DeLuca*[1]*, Brad Goodwin*[2]*, Peter Kowalski*[2]*, Karen Scruton*[2]*, Kent Thomas*[1] *and Elaine Cohen Hubal*[1]*, (1) U.S. Environmental Protection Agency, (2) Centers for Disease Control and Prevention*

Per- and polyfluorinated substances (PFAS) are persistent anthropogenic chemicals that have been widely used for their water-, stain, fire-, and stick-resistant properties and are persistent in the environment and human body. They have also been linked to adverse health effects such as liver and kidney disease, decreased fertility, developmental impacts, and cancer. The Agency for Toxic Substances and Disease Registry (ATSDR) conducted exposure assessments in eight U.S. communities with a history of contaminated groundwater due to the use of aqueous film forming foams at nearby military installations. Although drinking water contamination was mitigated in these communities over two years prior to the assessment, blood serum levels of some PFAS were elevated compared to the general population, which may be explained by the long half-lives of many PFAS. However, PFAS exposure can also occur via non-drinking water pathways, such as ingestion, inhalation, and dermal contact with house dust, which is an aggregate of chemicals present in the residential environment. Here, we analyze ATSDR's exposure assessment data for seven PFAS for a subset of 201 participants with paired household dust and blood serum samples. Across all communities, perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) were detected in the dust of 88% and 85% of households, respectively. Perfluoroundecanoic acid (PFUnA) was detected the least frequently in dust, having been detected in only 46% of households. Geometric mean dust concentrations ranged from 11.8 ng/g for PFOS to 2.2 ng/g for PFUnA, although there was significant variation between communities for three of the seven PFAS. This presentation will explore the relationship between PFAS levels in participants' blood serum and PFAS measured in their household dust, using hierarchical linear models. Additionally, we will examine associations between participants' responses to a questionnaire and PFAS levels in their household dust. We expect that the results will build the understanding of how exposure via household dust contributes to total PFAS exposure in impacted communities and what factors drive PFAS contamination in the residential environment. Disclaimer: The views expressed in this presentation are those of the author(s) and do not necessarily represent the views or the policies of the U.S. Environmental Protection Agency or the Agency for Toxic Substances and Disease Registry.

**4.12.T-02 Pesticides Concentrations in Canadian Low-Income Homes**

***Sara Vaezafsh**[1], Liisa Jantunen[2], Jeffrey Siegel[1] and Miriam L. Diamond[1], (1) University of Toronto, Canada, (2) Environment and Climate Change Canada, Canada*

Pesticides in residential buildings originate from intentional use to control pests either in the living environment or on pets, construction materials, treated consumer products, and from outdoors. Pesticide exposure can pose health risks, leading to restrictions in many countries of either total or indoor use of organochlorine, organophosphorus, and carbamate pesticides. The current-use pesticides pyrethrins and pyrethroids, are widely used worldwide for domestic applications. Many pesticides are highly persistent in indoor environments. Humans exposures are of particular concern for racialized and marginalized populations, such those of low socio-economic status (SES), who may live in low quality housing where pesticide use is more prevalent than in higher quality housing.

To the best of our knowledge, only one study in Canada published on 2018 has measured certain organochlorine pesticides in 20 homes but did not evaluate households with low SES. Our study aimed to measure concentrations of legacy and current used pesticides in the indoor air of low SES households. We sampled the indoor air particulate matter (PM) of 46 residential units in seven multi-unit residential buildings with low SES using portable air cleaners equipped with high-efficiency filters during winter 2017. These buildings were built in Toronto, Canada in the 1970s, prior to the discontinuation of DDT and heptachlor registration.

In 91% of samples, at least one pesticide was detected, including both current and legacy pesticides. Among the class of pyrethroid insecticides allethrin, permethrin, and tetramethrin were found to have the highest levels. While chlorothalonil and *p,p*-DDT were banned from domestic use in Canada in 1985 and 2011 respectively, they had detection frequencies (DF)>30%. Non-metric multidimensional scaling showed the association between tobacco smoking and certain pesticides including chlorothalonil, dazomet, permethrin, pyriproxyfen, pyrethrin I, and pendimethalin. These pesticides had DF>60% in units with evidence of smoking. The finding of similar pesticides profiles according to the specific building (e.g., permethrin with DF>70% in some buildings) suggested pest eradication programs implemented throughout the building-by-building management.

Detection of banned pesticides in units verified the persistence of organophosphorus and organochlorine pesticides. ***Integrated Pest Management*** should be used to minimize application rates of pesticides.

**4.12.T-03 Indoor and Personal PM2.5 Samples Differ in Chemical Composition and Alter Zebrafish Behavior Based on Primary Fuel Source**

***Shayla Victoria***[1]*, Lisandra Trine*[2]*, Staci Simonich*[2]*, Perry Hystad*[2] *and Courtney Roper*[1]*, (1University of Mississippi, (2) Oregon State University*

Fine particulate matter ($PM_{2.5}$) is the mixture of solid and liquid components of air pollution that are 2.5 µm or smaller in aerodynamic diameter. $PM_{2.5}$ exposure has been found to impact multiple systems of human health, including the cardiovascular and respiratory systems, while animal models have recently exhibited developmental impacts upon exposure. However, little is known about the potential health impacts of $PM_{2.5}$ generated from different indoor fuel sources and how exposure differs between personal samples and stationary samples in a household. $PM_{2.5}$ was collected using one stationary sampler in the kitchen and two additional samplers, worn by females and males, in homes in Kheri, India that used either biomass or liquified petroleum gas (LPG) as primary fuel sources. $PM_{2.5}$ filters were pooled by fuel type and monitor placement, resulting in 6 groups. Samples were analyzed for chemical composition, including elements and 125 organic compounds (parent-, hydroxy-, oxy-, nitro-, high molecular weight-, and heterocyclic-polycyclic aromatic hydrocarbons (PAHs)), and oxidative potential. Zebrafish (*Danio rerio*) embryos, a common developmental model for human health, were exposed to varying concentrations of collected $PM_{2.5}$ beginning at 6 hours post-fertilization until 5 days post-fertilization and behavioral analyses were conducted. Detected $PM_{2.5}$ concentrations exceeded World Health Organization daily exposure guidelines by 5-15 times. PAH concentrations were greater in biomass samples than LPG samples, while the highest percentages of carcinogenic PAHs were observed in the biomass samples collected from the kitchen and the males, 15% and 10% of the total quantified PAHs, respectively. Zebrafish exposed to $PM_{2.5}$ experienced greater incidence of mortality, morphological changes, and altered behaviors, including the embryonic- and larval photomotor responses. In addition to the detection of relatively high $PM_{2.5}$ concentrations, $PM_{2.5}$ varied in chemical composition, especially in compounds linked to human health impacts, based on fuel type and monitor placement. Notably, biological responses were elicited by all sample types, despite the current understanding that LPG is an adequate alternative for biomass fuels. Further research is needed to determine the chemical components and safety of indoor fuel sources.

**4.12.T-04 Composition of Per- and Polyfluoroalkyl Substances (PFAS) in House Dust from United States Homes**

***Nicole DeLuca****, Jeffrey Minucci, Kelsey Miller, Myriam Medina-Vera, Karen Bradham, James McCord and Elaine Cohen Hubal, U.S. Environmental Protection Agency*

House dust can be an aggregate of harmful chemicals present in residential environments and an important human exposure route for per- and polyfluoroalkyl substances (PFAS). The composition of PFAS chemicals in house dust can be a function of the home's proximity to point sources or different household materials (i.e., consumer products, furnishings, carpeting, cookware, clothing, and cleaning products) in the home. The U.S. Department of Housing and Urban Development (HUD) conducted the first survey of residential hazards called the American Healthy Homes Survey (AHHS) in 2005, which was followed by a second survey (AHHS II) in 2017. The U.S. Environmental Protection Agency (EPA) collaborated with HUD on both of these efforts and received subsequent OMB approval to analyze PFAS in house dust collected during the AHHS II study. While not considered nationally representative, the AHHS II house dust samples were collected in many regions throughout the contiguous U.S. and Hawaii. We analyzed ~250 house dust samples from homes in 37 states for 16 PFAS chemicals (PFOA, PFOS, PFNA, PFNS, PFHxS, PFHxA, PFBS, PFBA, PFDA, PFDoA, PFDS, PFHpA, PFHpS, PFPeA, PFPeS, PFUnDA). All targeted chemicals were detected in the house dust samples, with detection rates ranging from 9% for PFNS to 97% for PFOA. Eight chemicals (PFOA, PFNA, PFOS, PFHxS, PFDA, PFUnDA, PFDoA, and PFHpA) were measured above the limit of detection in at least 60% of house dust samples. Overall, the highest median concentrations observed in house dust were PFOS (9.07 ng/g) and PFOA (8.57 ng/g). The highest maximum concentrations measured were PFDA (4275.80 ng/g) and PFOA (4067.80 ng/g). Questionnaire and geographic information collected alongside the house dust samples will be analyzed to investigate variability in the composition of PFAS chemicals in house dust related to demographics, behaviors and activities, housing characteristics, and proximity to point sources. Disclaimer: The U.S. Environmental Protection Agency (EPA) through its Office of Research and Development funded and managed the research described here. The views expressed in this abstract are those of the author(s) and do not necessarily reflect the views or policies of the EPA.

**4.12.T-05 Evaluation of Cosmetics in Commerce in Canada for Perfluoroalkyl Acids their Precursors**

***Amila O. De Silva*** *and Cassandra Brinovcar, Environment and Climate Change Canada*

Per and polyfluoroalkyl substances (PFAS) have been extensively used in industrial and commercial applications. Substances with consecutive CF2 groups belong to the classic PFAS categories: perfluoroalkyl carboxylic acids (PFCA), perfluoroalkyl sulfonic acids (PFSA) and their precursors. Robust analytical methods are well established for PFCA and PFSA, particularly the C4 to C14 chain lengths. Given the wide range and diverse identity of precursors in commerce, their analysis remain largely unchartered. Earlier work has demonstrated the presence of PFCA, PFSA and one class of PFCA-precursors known as polyfluoroalkyl phosphate esters (PAPs) in North American and European cosmetics. Earlier experiments evaluated organofluorine content in cosmetics using total extractable organofluorine methods through combustion ion chromatography or total fluorine using particle-induced gamma-ray emission. In our research we implemented the total oxidizable precursor (TOP) assay to cosmetics purchased in Canada wherein hydroxyl radicals are produced in solution to transform precursors to PFCAs. We applied a direct TOP assay to cosmetics without the use of a sample extraction prior to oxidation. The oxidized product was then subjected to weak anion exchange solid phase extraction to measure PFCA and PFSA content using Orbitrap mass spectrometry. Using this approach we determined the post-oxidation perfluoroalkyl acids in 10 samples of cosmetics purchased in 2021. In our results, we found the product labeling to be consistent in so far as the presence of an organofluorine ingredient in the package label translated to PFCA concentrations above the blank in oxidized products. The oxidized product of both eyeliners, which contained the ingredient perfluorononyl dimethicone contained high concentrations of PFBA, and PFPeA. The oxidized of one BB cream containing perfluorooctyl triethoxysilane also had high concentrations of PFBA, PFPeA, PFHxA, and PFHpA while another BB cream brand did not appear to have precursors, consistent with its product labeling. One of the face powders containing C9-15 fluoroalcohol phosphates, had high oxidized concentrations of PFBA and all chain lengths up to PFHxDA. However, the other face powder, containing dimethiconol fluoroalcohol dilinoleic acid, did not appear to have C4 to C16 precursors. Our results show PFCA-precursors are in cosmetics in Canada but also that certain products used for the same application are seemingly PFCA-free.

**4.12.T-06 Uptake of Precursors and Legacy Per- and Polyfluoroalkyl Substances (PFASs) into Root, Shoot, and Fruit Crops from Aqueous Film-Forming Foam Impacted Water and Soil: Human Health Exposure Implications**

***Juliane Brown*[1]**, *Sarah Doyadora[2], Pingping Meng[2], Owen W. Duckworth[2], Detlef Knappe[2] and Christopher Higgins[1], (1) Colorado School of Mines, (2) North Carolina State University*

There is limited information on uptake of per- and polyfluoroalkyl substances (PFAS) into food crops from contaminated irrigation water and soils. Assessing human-health risk from consuming PFAS-impacted food crops requires quantifying the transfer of these compounds in the environment into edible plants. Published plant uptake bioaccumulation data are mostly limited to a subset of individual perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs) with few having more than one uptake factor determined for one or a few food crops, and uptake data into food crops from water and soil impacted by aqueous film-forming foam (AFFF) containing PFAS have not been previously reported. Assessing human health risk from exposure to AFFF-impacted food crops requires determining which precursors and legacy PFAS may pose the greatest exposure risk to consumers in different types of food crops (root, shoot, and fruit).

Greenhouse-based plant uptake experiments using AFFF-spiked irrigation water or spiked soils were conducted and plants were harvested at maturity and separated into plant compartments. Differences in uptake between legacy and precursor PFAS in an AFFF mixture were examined in plant compartments for radish, lettuce, and tomato crops. Analyses were done using liquid chromatography quadrupole time-flight mass spectrometry using targeted, suspect screening, and non-target analysis of the samples. Bioaccumulation patterns of PFCAs and PFSAs in these crops and plant compartments generally follow previously reported patterns of chain length dependency. Initial screening of suspect data indicate bioaccumulation of PFAS precursors from AFFF-spiked water into food crops is limited, possibly due to sorption to soil particles or metabolic transformation. Suspect screening and non-target analysis of the water, soil, and plant data will be used to provide insights into bioaccumulation patterns of PFAS precursors found in an AFFF mixture. Semi-quantitative analysis will be applied to suspect matched compounds to estimate bioaccumulation factors. Based on these findings, exposure risk from consuming AFFF-impacted agricultural produce will be evaluated.

**4.12.T-07 Consumer Exposure to Emerging Chemicals in Seafood Based on Customer Choice, Accessibility, and Availability**

***Megha Bedi*** *and Carla A. Ng, University of Pittsburgh*

Diet is a major route for exposure to organic contaminants, a substantial portion of which comes from seafood. Given increasing global seafood consumption rates, there is a proliferation of seafood products on the market, including more expensive options such as those labelled “sustainable.” However, a critical factor that is still largely unknown is whether choices made by consumers while buying seafood might also translate to differences in chemical exposures. Here, we investigate levels of contaminants of emerging concern in seafood consumed by local communities in Pittsburgh and assess if exposures vary according to where and what customers shop. We surveyed food markets across stores in the city best representative of the variability in prices, labels, geographical origins, and production methods (e.g. farmed vs. wild) to select a set of 46 fish and shellfish samples. Samples were screened for a wide suite of current-use and emerging chemicals, including antibiotics, pesticides, and per- and polyfluorinated alkyl substances (PFAS) using high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) and low-pressure gas chromatography tandem mass spectrometry (LPGC-MS/MS). Measured concentration were then used to build exposure estimates and evaluate trends that consider different diet scenarios (e.g. based on cost, cultural preferences, and neighborhood availability).

**4.12.T-08 Silicone Wristbands as Personal Passive Sampling Devices: Current Knowledge, Recommendations for Use, and Future Directions**

***Samantha Samon*[1,2]*, Stephanie Hammel*[3]*, Heather M. Stapleton*[2] *and Kim Anderson*[1]*, (1) Oregon State University, (2) Duke University, (3) The National Research Centre for the Working Environment, Denmark*

Personal chemical exposure monitoring is necessary to determine the frequency and magnitude of individual chemical exposures, especially since exposure to some chemicals has been associated with adverse health outcomes. In the last decade, silicone wristbands have emerged as a new chemical exposure assessment tool and have since been utilized for assessing personal exposure to a wide range of chemicals in a variety of occupational and non-occupational settings. Advantages of utilizing silicone wristbands as passive sampling devices include capturing individual level exposure, quantifying exposure to chemical mixtures, and minimalizing participant burden to support sample collection. However, as their popularity grows, it is crucial that they are used in the appropriate context and within the limits of the technology.

Silicone wristbands provide an estimate of integrated time-averaged exposure and capture both inhalation and dermal exposure routes. Most studies utilize wristbands for assessing exposure to semi-volatile organic chemicals such as pesticides, flame retardants and PAHs. In a growing number of studies, wristbands have been shown to be positively and significantly correlated with exposure biomarkers in blood and urine, and are equivalent or better than contemporary exposure assessment tools such as sampling house dust or active air samplers. These studies provide evidence that wristbands can be used in epidemiology studies. For instance, Hoffman et al., demonstrated that wristbands are just as good or better than urine samples in monitory exposure to the flame retardants TCPP and TDCPP, which strengthens the Poutasse et al., 2019 finding that higher levels of TDCPP found in silicone pet tags worn by cats were associated with feline hyperthyroidism.

Lastly, based off of the existing literature surrounding silicone wristbands several considerations should be made when using these tools in exposure assessment studies. These include where the wristband is worn on the body (e.g wrist, ankle, chest, etc.), duration of deployment, physical-chemical properties of target chemicals, and the end goal for the exposure assessment data. While more research is necessary to solidify silicone wristbands as a premier chemical exposure assessment tool, current evidence suggests that silicone wristbands are a useful tool while measuring personal chemical exposure and have the potential to better characterize the external exposome.

**4.12.P Human Exposure to Organic Contaminants of Concern**

**4.12.P-We123 Determining Background Concentrations and Associated Human Health and Ecological Risks of PFAS in Massachusetts Soils**

***Lisa M. McIntosh**, Woodard & Curran*

In the context of hazardous waste site investigation, it is critical to understand the background contribution of constituents to determine whether a regulated release to the environment has occurred, to understand the nature, extent and risks associated with release-related constituents, and to identify appropriate cleanup levels for remediation. This concept of background is especially relevant to PFAS, a ubiquitous contaminant in the environment for which the Massachusetts Contingency Plan (MCP) has promulgated stringent criteria for soil. However, in Massachusetts, as in many states, background levels of PFAS in soil are currently not established.

This study was created to quantify typical background levels of 36 PFAS in soils across Massachusetts. The study included the collection of 100 surface soil samples (0 to 6 inches below ground surface) from locations across Massachusetts in undeveloped, recreational, conservation lands that were not in proximity to known PFAS releases or potential source locations. Analytical results showed measurable concentrations of nine PFAS in soil across all regions of the state, and in many samples, at levels exceeding MCP default standards that are based on potable water use of an underlying aquifer. Based on these data, we evaluated the human health and ecological risks associated with exposure to background concentrations of PFAS in soil, and found that direct contact with PFAS concentrations in a recreational setting was unlikely to pose a significant human health risk, and that generally, PFAS levels were below effects-based levels for invertebrates and plants; however, there are uncertainties associated with risk to higher trophic level receptors due to the potential for biomagnification.

Understanding background levels of PFAS and their relative risk is critical in assessment of regulated releases that may warrant response actions. Results from this study will broaden our understanding of the levels and distribution of PFAS in Massachusetts soils, as well as the larger understanding of the ubiquitous presence of PFAS across our environment.

**4.12.P-We124 Screening Potential Drinking-Water Contaminant Effects Based on Point-Of-Use Exposures and Cumulative Ratios of Health-Benchmarks and In Vitro Activity Cutoffs**

***Paul M. Bradley**, Kristen M. Romanok and Kelly L. Smalling, U.S. Geological Survey*

The magnitudes, cumulative dose-response relations, and potential adverse outcomes of drinking-water exposures to complex mixtures of environmental contaminants of historical and emerging concern are global research priorities. Target assessments of mixtures of inorganic and organic contaminants in point-of consumption drinking water (tapwater) have been conducted to inform tapwater exposures and corresponding estimations of cumulative human-health risks across the US. More than 460 organic, 35 inorganic, and select microbiological contaminant indicators have been assessed in tapwater from home and commercial locations supplied by public community water systems and private-wells. Analytical results demonstrate that human exposures to inorganic and organic contaminant mixtures, which are rarely monitored together in drinking water at the point of consumption, are common. Concentrations of regulated and unregulated TW contaminants are used to calculate cumulative in vitro bioactivity ratios and Hazard Indices (HI) based on existing human-health benchmarks. Exceedances of human-health benchmarks and health-based HI screening levels of human-health interest are common to both public and private drinking water supplies. Study findings support further investigation of the potential cumulative risk to vulnerable populations and illustrate the importance of continued broad characterization of tapwater exposures using analytical capabilities that better represent the documented complexity of contaminant mixtures in ambient source waters. Increased public engagement in source-water protection and drinking-water treatment, including point-of-use treatment options, is warranted to reduce risks associated with long-term TW contaminant exposures, especially risks to vulnerable populations.

**4.12.P-We125 Direct Comparison of Contaminant Exposures and Potential Risk in Public and Private Tapwater in a Sole-Source Aquifer Setting**

***Kristen M. Romanok****, Kelly L. Smalling and Paul M. Bradley, U.S. Geological Survey*

In the United States (US), federal and state agencies regulate and monitor public-supply drinking water while private-supply monitoring is rare. Insufficient comparable information on contaminant-mixture exposures and risks between private- and public-supplies undermines tapwater (TW) consumer decision-making. We compared private- and public-supply residential point-of-use TW at Cape Cod, Massachusetts, where both supplies shared the same groundwater source. TW from 10 private- and 10 public-supply homes was analyzed for 487 organic, 38 inorganic, 8 microbial indicators, and 3 *in vitro* bioactivities. Concentrations were compared to existing protective health-based benchmarks, and aggregated Hazard Indices (HI) of regulated and unregulated TW contaminants were calculated along with ratios of *in vitro* exposure-activity cutoffs. Seventy organic and 28 inorganic constituents were detected in TW. Median detection counts were comparable between public- and private-supply samples, but median cumulative concentrations were substantially higher in public supply due to disinfection byproducts in six locations with chlorine-disinfected TW and corresponding lower heterotrophic plate counts. Concentrations equivalent to Environmental Protection Agency maximum contaminant (nitrate) and treatment action (lead and copper) levels were exceeded only in private-supply TW samples. Exceedances of health-based HI screening levels of concern were common to both public and private TW supplies. Results indicate comparable cumulative human-health risk from diverse contaminant exposures in private- and public-supply TW in a shared source-water setting.

**4.12.P-We127 A Science-Based Approach to Addressing the Health and Environmental Safety Issues of PFAS**

***Annaleise Jane Conway***[1]*, Lisa Tolbert*[1] *and Christina Ross*[2]*, (1) Integral Consulting Inc., (2) Credo Beauty*

Per- and polyfluoroalkyl substances (PFAS) have become ubiquitous in the environment and therefore have garnered increasing attention from private companies, academia, lawyers, and regulators. PFAS have most commonly been associated with firefighting foam, clothing, furniture, food packaging, and heat-resistant nonstick cookware. However, PFAS are utilized as ingredients in an even wider variety of consumer products. Recently, cosmetics and personal care products have been identified as potential sources of PFAS leading to exposure for humans and the environment. The current labeling requirements in the United States, which allow for low levels or undisclosed "incidental" ingredients, along with supply chain contamination issues, mean that even products that do not explicitly state PFAS as ingredients may in fact contain PFAS. This revelation has led to concerns that other exposures to PFAS (e.g., through use of cosmetics and personal care products) may lead to toxic effects in humans and the environment. This presentation discusses the toxicity of PFAS to humans and the environment as well as the current understanding of PFAS in cosmetics including recent literature, litigation activities, and regulatory actions, and presents potential paths forward for industry, scientists, and the general public regarding this emerging concern to gain a better understanding of the current landscape and increase the applicability of data moving forward.

**4.12.P-We129 Considerations for Scientists and Firefighters when Selecting a Sample Type**

***Emily Bonner** and Kim Anderson, Oregon State University*

The occupational health of structural firefighters is a vital area of study due to complex exposures to chemicals, particulate matter, heat, and stress during fire responses, and negative health effects associated with firefighting (e.g. cancer, cardiovascular disease). Studies on exposure, efficacy of exposure interventions, and health effects can all benefit firefighters by providing guidance on effective hygiene practices, policies, and evidence to propel the development of better personal protective equipment. Naturally, these studies involve firefighters as participants. However, some of the sampling methods in use are invasive or more challenging for firefighters to comply with after a fire, such as giving blood or carrying additional gear for air monitoring. We want to foster better communication between scientists and firefighters surrounding exposure sampling techniques to generate more realistic expectations surrounding sample collection and study results for both groups. This work has the potential to improve compliance in community-engaged studies, and ultimately, increase the quality of information regarding the reduction of firefighter exposures and disease rates. This presentation covers the work our lab has done to communicate with firefighters through publication in a non-academic trade journal with the intention of cultivating a better understanding of chemical exposure study tools. The article that we produced provides tools to help inform the most appropriate sample type and collection methodology based on the chemicals of interest. Generally, we considered two major categories of samples: external, such as air samples, or biological, such as blood or urine samples. Sample comparison tables provide a framework for discussion between scientists and firefighters when it comes to designing a study and recruiting participants. We also provide a case-study to highlight the importance of using a sampler that is fit-for-purpose and limits the burden on firefighters. Setting expectations with study participants about the level of involvement expected when providing samples, as well as the type of information they can expect to receive at the end of a study is key to the success of community-engaged research. Ultimately, it benefits participants and to make an informed decision on a sample type based on practicality for firefighters and its ability to produce meaningful data with the appropriate analysis.

**4.12.P-We130 Understanding PFAS Interactions With Kidney Function in Health and Disease**

***Shan Niu**[1], Yuexin Cao[1], Ruiwen Chen[1], Megha Bedi[1], Alison P Sanders[1], Alan Ducatman[2] and Carla A. Ng[1], (1) University of Pittsburgh, (2) West Virginia University*

Per and polyfluoroalkyl substances (PFAS) are ubiquitous in the environment due to their widespread use and high persistence. Epidemiologic and toxicological evidence supports PFAS exposure contributes to various adverse health endpoints including changes in kidney function. In animals, sex- and species-specific differences in elimination half-lives of PFAS have been linked to the activity of transporter proteins and polypeptides for organic anions expressed in the kidney. However, interactions of kidney transporters with PFAS are still not fully understood. Moreover, kidney disease alters the expression of renal transporters, which will further influence PFAS renal elimination. Little is known about the influence of kidney disease on PFAS toxicokinetics. In this study, we first reviewed the available literature to characterize an array of human renal transporters relevant to renal elimination and reabsorption on the basis of their ability to transport PFAS and their expression levels relative to kidney function. An existing physiologically based pharmacokinetic (PBPK) model for perfluorooctanoic acid (PFOA) in male rats was used to explore how changes in transporter expression levels, glomerular filtration rates (GFRs), and serum albumin concentrations during kidney disease could impact PFOA half-life. Serum PFOA terminal half-lives were longer in rats with decreased levels of kidney efflux transporters (organic anion transporters Oat 1 and 3) and lower GFR compared to healthy rats, whereas serum PFOA half-lives were comparable between rats with 70% decreased albumin concentrations and healthy rats. However, initial half-lives were shorter in rats with decreased albumin. Additionally, the model shows that the influence on PFOA toxicokinetics is higher due to changes in GFR than changes in transporter expression levels. However, more information is needed to understand differences between rats and humans in order to translate these findings. Moreover, little information is available on the kinetics of renal transport of emerging PFAS. To this end, we also investigated the uptake rates of PFAS by renal transporters *in vitro* using cell-based assays. We focused on two emerging PFAS (GenX and F53B) to address current exposure concerns. This work provides up-to-date knowledge of how kidney function affects PFAS elimination, which is critical to setting evidence-based health advisory limits for these ubiquitous chemicals.

**4.12.P-We132 Molecular Evaluation of Mammalian Neurotoxicity Responses following Inhalation of Neat and Ethyl-Parathion-Incorporated Dust**

***Saroj K. Amar***[1]*, C.P. Gut*[2]*, R.M. Styles*[1]*, R.J. Johnson*[2]*, D.M. Holtzapple*[2]*, J.L. Stricker*[2]*, S.M. McInturf*[2]*, E.A. Phillips*[2]*, K.L. Mumy*[2]*, D.R. Mattie*[3]*, Mark Chappell*[1] *and Kurt A. Gust*[1]*, (1) U.S. Army Engineer Research and Development Center, (2) Naval Medical Research Unit Dayton, WPAFB, (3 )Air Force Research Laboratory*

Organophosphorus pesticides (OP), including ethyl-parathion (EP), inhibit acetylcholinesterase (AChE) activity causing neurotoxic effects in mammals. EP is commonly used in agricultural applications where adsorption onto soil represents a potential secondary exposure source via inhalation of resuspended dust. Thus, we investigated 4 hour acute inhalation exposures to neat EP (1, 10 and 20 $mg/m^3$) and dust-incorporated EP (0.0095, 0.09 & 0.185 $mg/m^3$) followed by 2, 24, and 48 hour recovery periods in Sprague Dawley rats. Molecular responses were investigated from at least 3 rats per exposure. Hippocampal transcript expression of AChE and its nicotinic receptors nAChRα4 and nAChRβ2 were significantly decreased ($p \leq 0.05$) in rats exposed to neat and dust-incorporated EP. AChE activity was significantly decreased in hippocampal tissues harvested from animals exposed to neat and dust-incorporated EP, consistent with the known OP mechanism of action. Significantly increased transcriptional expression for TNF-α, IL-1B, and IL-6, which are recognized neuro-inflammatory response elements, was observed in hippocampus of rats exposed to neat and dust-incorporated EP, likely via over-stimulated nicotinic acetylcholine receptors. Additionally, a significant increase in malondialdehyde (MDA) levels, indicative of lipid peroxidation, was observed in tissues from rats exposed to the EP-incorporated dust. Nox2 transcriptional expression significantly increased in rats exposed to neat EP exposures where *Nox2*-derived reactive oxygen species might have caused the lipid peroxidation. Alternatively, significantly decreased transcriptional expression of SLC3A2 and SLC7A11 in rat hippocampus from the dust-incorporated EP exposure suggest a putative role of nicotinic acetylcholine receptors in iron homeostasis and possible ferroptosis-related responses. In support of this, ELISA-based ferritin measurements indicated significantly increased ferritin in tissues harvested from both neat and dust-incorporated EP exposed rats. However, ferric iron levels in hippocampi were not significantly increased and ferrous iron had a non-monotonic relationship with EP concentrations. Finally, the EP concentrations in dust were two orders of magnitude less than the neat exposure, yet were similarly affective for most endpoints described above. This observation indicates that EP adsorbed to dust may represent an unexpected and potentially more toxic exposure risk.

## 4.12.V Human Exposure to Organic Contaminants of Concern

**4.12.V-01 PFAS Exposure via Drinking Water and Diet in a Midwestern Community with a Former Paper Mill**

***Ying Guo***[1]*, Rachel Bauer*[1]*, Ankita Bhattacharya*[1]*, Heather M. Stapleton*[2]*, Christopher Higgins*[3]*, John Adgate*[4] *and Courtney Carignan*[1]*, (1) Michigan State University, (2) Duke University, (3) Colorado School of Mines, (4) Colorado School of Public Health*

Drinking water is an important exposure pathway when PFAS concentrations are elevated whereas for the general population diet is believed to contribute the most exposure. Local and homegrown foods may also be an important exposure pathway for some communities. Therefore, we conducted a detailed biomonitoring study for a Midwestern community with historic (pre-2018) PFAS drinking water contamination (1600 ppt). We enrolled 129 participants from 92 homes, administered dietary surveys, collected samples of serum and drinking water, and collected data on historic drinking water concentrations. We investigated 48 PFASs in water from private wells and found elevated concentrations of nine more not previously identified. Serum concentrations were elevated compared to the general population for PFOS, PFOA, PFHxS, PFHpS and NMeFOSAA. We will share our findings of relative contributions of serum PFASs from the drinking water and dietary exposure pathways under our hypothesis that historic drinking water is the strongest predictor in this population followed by local and other foods.

**4.12.V-04 PFAS Exposure from Home Produced and Locally Captured Foods in a Midwestern PFAS-Impacted Community**

***Ankita Bhattacharya***[1], *Sarah Choyke*[2], *Juliane Brown*[3], *Christopher Higgins*[3], *Heather M. Stapleton*[4], *Courtney Carignan*[1] *and Rachel Bauer*[1], *(1) Michigan State University, (2) Eurofins Environment Testing (EET), (3) Colorado School of Mines, (4) Duke University*

Dietary exposure to poly- and perfluoroalkyl substances (PFASs) has been raised as a concern among communities with PFAS-impacted water. Elevated concentrations have been identified in livestock and wildlife as well as soils and produce from home gardens in impacted areas. While elevated PFASs in drinking water clearly contribute to elevated exposures, less is known about the contribution of home produced and locally captured foods. Therefore, we conducted a detailed exposure assessment for a Midwestern community with historic (2018) drinking water contamination. We enrolled 129 participants from 92 homes, administered detailed exposure questionnaires, and collected samples of produce and soil from home gardens, eggs from home raised chickens, and venison from locally captured deer. Samples were tested for 48 different PFASs including PFOA and PFOS, and non-targeted suspect screening was done on a subset of soil samples. Elevated concentrations of PFOS were found in eggs (3.5 ppb) and venison (14 ppb). PFOS was detected in all soil samples (maximum=1.8 ppb) but not produce. Produce contained low concentrations of other PFASs (<0.5 pbb) with the highest detection frequencies for PFHxS and 4:2 FTS (50%) followed by PFBS, PFOS, PFBA, and PFPeA (>30%). In addition to sharing details of these findings we will report exposure estimates for home produced and locally captured foods using site-specific PFAS concentration and consumption frequency data.

**4.12.V-05 High Ocurrence of Aflatoxins in Maize and Peanuts and Exposure Assessment of the Population in Ghana**

***Enock Dankyi***, *Richard Boadu Opoku and Dorcas Osei-Safo, University of Ghana*

Aflatoxins are widely recognized as highly carcinogenic mycotoxins and important natural contaminants of a wide range of crops, particularly maize and peanuts which constitute integral components of staple diets in many developing countries including Ghana. Aflatoxin contamination is widespread in Africa and poses huge public health risks due to its high presence in food and feed. Notwithstanding the widespread exposure, research data and knowledge on aflatoxins in food and exposure to the population remains low, and largely restricted to exported food products, leaving millions exposed to potentially significant levels of the toxins. In this study, we report a comprehensive analysis of aflatoxin content (AFB1, AFB2, AFG1, AFG2) in 303 samples comprising 165 samples of maize and 138 samples of peanuts from farms, homes, markets, and storage centers in eight regions across Ghana. We found that, aflatoxins were present in 81% of maize samples with concentrations ranging from 0.20 to 1129.7 µg/kg. In peanut samples, aflatoxins were present in 74% of samples with concentrations from 0.20 to 1242.9 µg/kg. Aflatoxin B1 occurred in the highest concentrations in both crops with an average concentration of 119.8 µg/kg and 71.8 µg/kg in maize and peanuts, respectively and were present in 49% of maize and 25% peanut samples at concentrations that exceed the Ghanaian standard of 10 µg/kg. Our study shows significant exposure of aflatoxins to the Ghanaian population with some of the highest concentrations and prevalence rate being reported in the country. This study will help inform policy on prioritization of aflatoxins as a major food safety concern in Africa, in line with the recent establishment of the Partnership for Aflatoxin Control in Africa (PACA) by the African Union Commission.

**4.13 In the Neighborhood and Out to Sea: Shedding Light on Tire Wear Microplastics**

**4.13.T-01 How Much Tire Rubber Do Vehicles Shed? California USA Estimates and Their Implications**

***Kelly Moran****, Alicia Gilbreath, Miguel Alexander Mendez, Diana Lin and Rebecca Sutton, San Francisco Estuary Institute*

Every vehicle on the road sheds tiny particles from its rubber tires into the environment. Modeling studies suggest that tire wear may be one of the top sources of microplastics to the environment globally. As they disperse into the environment, tire particles convey tire tread ingredients into the air, into runoff, and eventually into surface waters. San Francisco Bay Area monitoring found that tire particles are the single most common microplastic flowing into the Bay, likely because stormwater runoff carrying these particles rarely receives treatment before it flows into local waterways. While multiple publications since the 1970s contain estimates of US tire particle emissions (ranging from 3.0 – 5.5 kilogram/year per capita), these estimates are based on various assumptions or extrapolations with roots in non-US data unadjusted for vehicle size. Due to the popularity of light trucks and wider roadways, US vehicles and their tires are larger, on average, than the European vehicles that underlie most emissions estimates. Using tire sales and measured tire particle emissions data from reliable sources, tire size adjustments as necessary, and local vehicle miles traveled data, we have estimated annual total and per-capita vehicle tire particle emissions in California USA (statewide) and in the San Francisco Bay area. We used two approaches: (1) the emission factor/mileage method and (2) the sales/tread-loss method. The estimates reveal the relative amount of tire wear emissions from various vehicle types, thereby informing priorities for mitigation actions like installation of on-vehicle tire wear debris collection systems. Additionally, we compared the annual tire wear particle emissions estimates to monitoring-based estimates of annual tire-wear particle flows into San Francisco Bay to obtain insights into the transport of particles between on-road emissions and aquatic habitat.

**4.13.T-02 Abundance and Distribution of Tire and Road Wear Particles in the Seine River, France**

***Timothy R. Barber***[1]*, Allison Dillon*[1]*, Sophie Claes*[1]*, Sharlee More*[2]*, Stephanie A. Thornton*[2]*, Kenneth Unice*[2]*, Steffen Weyrauch*[3]*, Thorsten Reemtsma*[3] *and Jos van Gils*[4]*, (1) ERM, (2) Cardno ChemRisk now Stantec, (3) Helmholtz Centre for Environmental Research – UFZ, Germany, (4) Deltares, Netherlands*

Tire and road wear particles (TRWP) are formed at the frictional interface between tires and the road surface, and this mixture of tire tread and road pavement material can wash from the road surface into receiving water bodies during precipitation events. Based on the density of TRWP, they are expected to partition into sediment, but the paucity of mass-based measurements has limited the knowledge on environmental concentrations. Surface water and sediment samples were collected from the Seine River in France with the objective to collect reliable measurements of TRWP. Sampling was conducted along 400 km of the Seine River between 31 May and 19 June 2021. Sample locations were established upstream of Paris, within the Paris metropolitan area, downstream of Paris near smaller urban areas, adjacent to an undeveloped area and near the confluence of the river and the English Channel. Surface water and sediment samples were collected from the left and right banks at each of the eight locations for a total of 18 samples, including two duplicate samples. In addition to direct river sampling, three sediment traps were deployed between 7 September and 9 November 2021 near the mouth of the river to quantify the flux of TRWP to sediment. Surface water retained solids and sediment samples were analyzed for TRWP by pyrolysis-gas chromatography/mass spectrometry (p-GC/MS) and estimated based on the zinc content as well as matrix characterization parameters such as total suspended solids, total and dissolved organic carbon and grain size. All retained solids and sediment samples measured using the ISO technical specification method (ISO/TS 21396) were reported below the TRWP detection limit. The detection limit was elevated due to matrix interferences, associated with naturally occurring organic matter, limiting the interpretation of these data. These samples were re-analyzed using a modified p-GC/MS method that minimized the matrix interferences in the samples and provided results that are more reliable. TRWP also was estimated by separating the sediment into the $<1.9$ g/cm$^3$ fraction and analyzing for zinc. Empirical factors were used to translate the zinc concentration to tire particle and TRWP concentrations. Comparisons are made between the various TRWP analytical methods and the matrix characterization parameters. In addition, the results are compared to available historical data. Potential causes for the observed spatial and temporal trends will be discussed.

### 4.13.T-03 Tire-Derived Contaminants in San Francisco Bay

***Ezra Miller***[1]*, Alicia Gilbreath*[1]*, Edward P. Kolodziej*[2]*, Kelly Moran*[1] *and Rebecca Sutton*[1]*, (1) San Francisco Estuary Institute, (2) University of Washington*

Tire particles are the most abundant type of microplastic entering San Francisco Bay via stormwater runoff. In California, urban stormwater runoff usually carries tire particles from roads directly to surface waters, through separate storm drain systems designed to prevent flooding and avoid sanitary sewer overflows. In addition to tire particles themselves, a number of potentially toxic tire-derived contaminants have been observed in Bay Area stormwater, including the preservative derivative known to be highly toxic to coho salmon, 6PPD-quinone. Previous monitoring of Bay Area stormwater indicated 6PPD-quinone is in stormwater at levels that are lethal to coho salmon. While coho salmon are now absent from Bay tributaries, steelhead (Oncorhynchus mykiss), a threatened species, are observed in some streams. New data indicate that rainbow trout, the freshwater version of steelhead, are also sensitive to this chemical. The Regional Monitoring Program for San Francisco Bay undertook a pilot monitoring effort to quantify a number of tire and roadway contaminants in Bay water samples collected following storm events to provide information on the impact of stormwater discharges on Bay contaminant concentrations. Results indicate these tire and roadway contaminants reach detectable concentrations in Bay water near stormwater discharge locations as well as in the center of the Bay, even with dilution from mixing. Furthermore, 6PPD-quinone reaches concentrations that may be of concern for steelhead both in the creeks during storms and within receiving waters post-storms.

### 4.13.T-04 Analysis of tire-related chemicals in fish fluids using liquid-chromatography couple with tandem mass spectrometer

***Denis da Silva****, National Oceanic and Atmospheric Administration (NOAA) Fisheries Northwest Fisheries Science Center*

6PPD [$N^1$-(4-Methylpentan-2-yl)-$N^4$-phenylbenzene-1,4-diamine] is a chemical used by the industry to protect the rubber in car tires, which makes this chemical widely present in urban stormwater runoffs. Only a few years ago, after a comprehensive investigation of stormwater runoffs and it's toxicity, WA State scientists determine that a quinone transformation product of the 6PPD was one of the key compounds responsible for the pre-spawn mortality of Coho Salmon (*Oncorhynchus kisutch)*. This recent finding triggered a wide number of studies worldwide in order to better understand the toxicity, environmental fate and analytical methods for measuring 6-PPD and its quinone derivative 6PPD-Q. However, most of the methods of analysis are focused on measuring 6-PPD-Q in water samples. We have been working on an analytical method for simultaneous analysis of 4 different tire-related chemicals, including 6PPD-Q, in fish samples, including plasma and bile. Our analytical approach includes sample extraction and cleanup using solid-phase extraction and final analysis using liquid-chromatography couple with tandem mass spectrometer technique. This method showed to be fast, relatively inexpensive and sensitive, with limit of quantitation around 10-100 pg/mL of sample. We intend to field validate this methodology using samples collected from urbanized and non-urbanized areas of Puget Sound and also determine the baseline levels of this exposure in the region. This analytical approach will not only help assess the environmental relevant exposure of fish to these toxic compounds, but will also provide important information while we advance on the understanding on fate and toxic mechanisms of these chemicals in fish.

### 4.13.T-05 Chronic Toxicity of Micro and Nano Tire Particles to Daphnia magna

***Brittany Cunningham****, Bryan J. Harper, Susanne M. Brander and Stacey L Harper, Oregon State University*

Monitoring of microplastics in aquatic environments has documented high levels of black rubber which are attributed to tire particles being generated through friction with the roadway. Though the impact of this flow of tire particles to the environment is not fully known, studies have shown that exposure to micro, and nano-sized particles, as well as tire leachate can be toxic to aquatic organisms. To date, most studies have focused on acute exposures, which are not as reflective of the length of real world environmental exposures. Chronic toxicity studies with tires are limited and have only been done using leachate alone or with much larger tire particles (<500 µm). There is a need to assess toxicity of tire particles in smaller size-classes to better understand the role that tire particle size has in conferring toxicity over longer periods of time. We investigated the impact of chronic exposure of *Daphnia magna* to micro (1-20 µm, 3.13 x $10^4$ – 2.5x$10^5$ particles/ml) and nano (<1 µm, 1.25 x $10^5$ – 1.0x$10^8$ particles/ml) sized tire particles. Triplicate exposures began at 6 days old and lasted for a total of 28 days. Mortality, reproduction, and molting were assessed daily, and growth was measured at the end of the exposure. Additionally, the F1 generation was reared for 28 days in tire-free water. Exposure to the highest micro and nano-sized tire particles resulted in mortality of *D. magna* before the end of the 28 day exposures. Where mortality was not observed, chronic exposure to the micro tire particles still had severe impacts, delaying, decreasing and even eliminating reproduction starting at 6.25 x $10^5$ part/ml. Chronic exposure to the nano tire particles had less severe impacts, but delayed and decreased reproduction at the second highest exposure level, 5.0 x $10^5$ part/ml. Impacts of tire-exposure in the parental generation were not seen to carry over into the F1 generation. Compared to acute exposures, much lower concentrations of tire particles in both micro and nano sizes caused mortality for chronically exposed *D. magna*. Furthermore, the immense reproductive effects that chronic exposure to tire particles have shown could have devastating impacts on populations of aquatic invertebrates.

**4.13.T-06 Fitness relevant impacts of tire particles and microfibers both alone and in mixture on euryhaline fish and invertebrates**

*Samreen Siddiqui, Sara J. Hutton, Emily Pedersen, Brittany Cunningham, Bryan J. Harper, Stacey L. Harper and* ***Susanne M Brander****, Oregon State University*

Plastic debris is a ubiquitous source of pollution in marine and estuarine ecosystems, with documented adverse health impacts on aquatic organisms. Although studies have been conducted across different polymer types, concentrations, and sizes (e.g., micro vs. nano), a gap remains in our knowledge of organism responses to environmentally relevant concentrations, particularly in estuaries where salinity can influence particle behavior and also potentially toxicity. Furthermore, little is known regarding combined effects of tire particles (TP) and polyester microfibers as an organic aquatic contaminant. Generated from automobile traffic, there is an estimated 1,121,000 t/a of TP in the United States alone, frequently detected in near urban areas, and fibers are the most frequently detected plastic type globally. As these particles aged in the environment under sunlight, their effects on organisms can also change. In this study we mimic such conditions in controlled environment to age the particle mixtures by exposing them to UV A, B, and C for 3 days. *Menidia beryllina* (Inland Silverside) and *Americamysis bahia* (mysid shrimp) were used as as indicator species to study the sublethal effects of environmentally relevant concentrations of synthetic TP-microfiber mixture in estuarine and bay environments. We exposed seven-day-old *A. bahia* (n = 9) and 5-7 days post fertilized *M. beryllina* to 3 concentrations of TP-microfiber mixtures in two size ranges (1- 20 µm and 80-120 µm respectively), at 15 ppt salinity for 28 and 21 days, respectively. A subset of fish and shrimp were used for behavioral analysis, while the rest were euthanized, preserved for growth and biochemical analyses. Results suggest that new TP-Microfiber mixture affected swimming behavior in *M. beryllina* and *A. bahia* that particles were internalized, and that growth was reduced in both species. In a similar experiment both *M. beryllina* and *A. bahia* were exposed to separately to TP and Polyester microfiber, where both the organisms demonstrated similar behavioral and growth effects. The presence of adverse effects in *M. beryllina* and *A. bahia* indicates that even at current environmental levels, which are expected to continue to increase, that coastal ecosystems experience impacts dependent on the physical properties of the plastic and associated chemicals.

**4.13.T-07 The Role of Stormwater Detention Ponds in the Transport of Tire Wear Particles to the Coastal Waters of South Carolina**

***Shannon Marie Bley***[1]*, John E. Weinstein*[2]*, Barbara Beckingham*[1] *and Joshua Robinson*[3]*, (1) College of Charleston, (2) The Citadel, (3) Robinson Design Engineers*

Microscopic tire wear particles (TWPs), shed from vehicular tire tread, are gaining attention as an emerging class of microplastic (MP) contaminants. TWPs are prevalent in coastal suburban and urban areas where they may be picked up and transported via urban surface runoff. This runoff may subsequently deposit TWPs in stormwater detention ponds, a type of best management practice designed to detain stormwater runoff to mitigate flooding whilst secondarily capturing contaminants. While TWPs and other MPs have been identified in water and sediment samples obtained from stormwater detention ponds, the extent to which they are transported into downstream natural receiving waters remains largely unknown. Previous studies in Charleston Harbor, SC (USA) have found a high proportion of TWPs among surface water and sediment MPs. The purpose of this study was to elucidate the role of stormwater detention ponds as a conduit of downstream TWP transport from upland sources to coastal waters. Water and sediment samples were collected from locations within 5 stormwater detention ponds and their receiving tidal creeks in Mount Pleasant, SC. Sediment samples underwent a density separation using a NaCl solution (1.2 g/cm$^3$) to extract lower-density TWPs and MPs, with subsamples undergoing a second separation using a sodium polytungstate solution (1.9 g/cm$^3$) to extract higher-density TWPs. Water and sediment samples were then sieved to two size fractions (500µm, 63µm) and chemically digested before being analyzed for MP (fibers, fragments, foams, films, spheres) and TWP abundance under a dissecting microscope. Suspected MPs and TWPs were confirmed as plastic by analyzing melt response using a hot needle test, with subsamples identified using ATR-FTIR. Preliminary results find decreasing TWP concentrations ($p=0.017$) and composition (% of total particles, MPs + TWPs) in water samples moving from pond inlets (38%) downstream into tidal creeks (<10%). Water MP concentrations were also found to differ between ponds according to dominant land use category ($p=0.01$). These results indicate that a majority of TWPs are settling out within the ponds, and that they are retained more effectively than other plastic particles. Forthcoming sediment results will be evaluated for similar trends. These findings will be used to advise stakeholders and coastal decision makers on future research and management decisions regarding stormwater pond efficacy to further reduce downstream TWP transport.

**4.13.T-08 Can Permeable Pavements Mitigate Environmental Emissions of Tire Wear Particles and Tire-Associated Contaminants?**

***Chelsea Mitchell*** *and Anand Jayakaran, Washington State University*

Tire wear particles (TWPs) contribute a substantial proportion of microplastics pollution and have recently been implicated as the source of 6PPD-quinone, a recently discovered chemical that is acutely toxic to coho salmon (*Oncorhynchus kisutch*). TWPs deposited on roads are transported to aquatic ecosystems via stormwater. Stormwater BMPs provide an opportunity to prevent TWP release, however little is known about the fate of TWPs or their associated contaminants in these systems. We conducted a series of experiments to quantify the treatment performance of 4 permeable pavement (PP) formulations for TWPs and 9 tire-associated contaminants including anticorrosion chemicals, rubber vulcanization accelerators, polymer cross-linking agents, and 6PPD-quinone. Experiments were performed on PP cells located in the driveway of a high school in Tacoma, WA. The PPs included concrete, asphalt, and equivalent concrete and asphalt cells amended with cured carbon fibers. Pavements were lined with plastic and outfitted with an underdrain pipe draining to an effluent sampling port. Pavements were dosed with municipal water via a custom sprinkler system covering a 32 m$^2$ area corresponding to the lined portion of the pavements for a 1-hr storm. During the 2$^{nd}$ experiment 500 g of 50 µm, cryomilled TWPs were applied to the dosing area of each pavement. The 1$^{st}$ and 3$^{rd}$ experiments served to establish a baseline for TWPs and associated chemicals and to evaluate the potential for continued release of these pollutants during subsequent storms. During each experiment, 13 flow-weighted effluent samples were collected for chemical analysis and microplastics extractions using an automated sampler. Chemical analysis was conducted using LC-MS/MS. TWPs were extracted from samples via density separation with $ZnCl_2$ and TWP concentrations were determined using image analysis on extracted filtrates. We hypothesize that PPs will attenuate the bulk of TWPs via settling and filtration. We expect concentrations of tire-associated chemicals to be independent of TWPs in effluents because of leaching of chemicals from particles retained in the pavements. Results from this study will help inform the treatment that can be expected for TWPs and associated chemicals with permeable pavements.

## 4.13.P In the Neighborhood and Out to Sea: Shedding Light on Tire Wear Microplastics

### 4.13.P-Mo115 The Influence of Environmental Factors on the Toxicity to Americamysis bahia from Tire Wear Particle Leachate

***April D. Reed***, *Brook F. Ashcraft, Ruth M. Sofield, Saeda P. Suhm, Leo S. Curtis, Jasper Conery and McKenna K Haney, Western Washington University*

Vehicle tires wear down and shed tiny particles, releasing them into the environment. Studies have found tire wear particles, which are made of natural and/or synthetic elastomer polymers, to contain many potentially harmful chemicals which can leach into water. The chemical toxicity of tire wear particle leachates and its implications to human and environmental health are of emerging concern. This study explores the influence of salinity, temperature, and leaching time on the toxicity of tire wear particle leachates to *Americamysis bahia*, a marine invertebrate commonly used in toxicity testing. The tires used for particle generation were sourced from two select urban areas, based on the Washington State Department of Health's Environmental Disparities Map, to compare the toxicity of tires from a tire shop in an environmentally impacted community to tires from a less environmentally impacted community. Using a factorial design, the tire wear particles were used to create leachates under eight combinations of temperature (10º C and 30º C), salinity (20 ppt and 30 ppt), and length of leaching time (2 and 14 days) for each tire group. Acute 96-hour toxicity tests on *A. bahia* were run using each leachate. Experimental results show higher toxicity with higher temperature and longer leaching time and little influence of salinity and tire source.

### 4.13.P-Mo116 Determining the Leaching Potential and Chemical Profiles of Tire Wear Particles in Aquatic Conditions

***Margaret Stack***, *Kelly Hollman, Natalie Mladenov, Kari Sant, Eunha Hoh and Alysia Daines-Ravn, San Diego State University*

Tire wear particles (TWP) are one of the most commonly identified microplastics in environmental water samples. However, limited data exists on the diverse chemicals that can leach from TWP. Our study aims to investigate the chemicals that leach from TWP in lab-created freshwater under various conditions, including size, time, and photoirradiation. We determined the chemical leaching rate of TWP using a total organic carbon analyzer and characterized the chemical profiles of leachate using solid phase extraction (SPE) to prepare the samples for non-targeted chemical analysis (NTA) with two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GCxGC/TOF-MS). Preliminary results using 10 g TWP/L show that most dissolved organic carbon (DOC) is released by 3 days of leaching, and small TWP (< 90 µm) leached higher concentrations of DOC than large TWP (~1 mm) (24.2 mg/L vs. 10.8 mg/L at 12 days of leaching, respectively). We detected known tire contaminants in both small and large TWP leachate, including 6PPD and hexa(methoxymethyl)melamine, both of which are predicted to be toxic. We also detected additional antioxidant-related quinolines, the suspected carcinogen aniline, and multiple bicyclic amines in the tire leachates. Further experiments will assess the impacts of UV radiation on TWP leachate in order to determine photodegradation rates and chemical transformation of leachate constituents. The results of this study will provide insight into the chemical risks of microplastic leachates and can be used to inform policy regarding the environmental health risks of plastic.

### 4.13.P-Mo117 Analysis of emerging automotive contaminants in environmental samples

*Karl Oetjen, Diana Tran and* ***Matthew Noestheden***, *SCIEX*

Recycled tire products pose risk to both the environment and human health. A recent study by Tian et al. (2021) identified the presence of a quinone transformation product of N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine (6PPD), as a potential source of acute mortality in U.S. Pacific Northwest Coho salmon

(Oncorhynchus kisutch). 6PPD is used as a tire rubber antioxidant and it, along with its transformation product 6PPD-quinone, were found in roadway runoff and stormwater samples at toxic concentrations. In addition to 6PPD-quinone, hexa(methoxymethyl)melamine (HMMM ), an additive in automotive (and other industrial) resins, has surged in public and academic research interest owing to their environmental prevalence and harmful impacts to wildlife. While roadway runoff is one pathway for tire related compounds to enter the environment, there are many other ways these compounds can be introduced. Recycled tire products have been used for a variety of purposes including landscaping, rubberized asphalt, and as a synthetic alternative for turf fields. These synthetic turf fields have been installed in the United States since the 1960s and currently, there are 12,000-13,000 synthetic turf fields nationally, with 1,200 – 1,500 new fields being installed each year. According to the United States Environmental Protection Agency (US EPA), 6PPD was observed in nontargeted analysis of both recycling plant and synthetic turf field samples with relatively high response area counts, along with many other compounds that were tentatively identified or not identified. Due to the wide variety of compounds present in tire derived samples and the presence of thousands of unknown features, comprehensive screening methods in addition to highly sensitive quantitation methods are required. This study utilized both a quantitative $MRM^{HR}$ method and a data independent acquisition approach (SWATH DIA acquisition) to acquire high-quality, high-resolution MS/MS data to screen for both known and unknown tire derived contaminates in water. Using this quantitative method, LOQs for 6PPD-quinone and hexa(methoxymethyl)melamine HMMM were determined to be 10ppt and 500ppt respectively. While the nontargeted method identified 17 other amide compounds in addition to 6PPD-quinone in tire impacted water samples. Of these 17, several compounds were not found in mass spectral databases or in the literature, as they are likely impurities created in the tire production process.

**4.13.P-Mo118 Atmospheric Deposition of Microplastics and Tire Wear Particles in Salt Marsh Habitats: Relationship to Meteorological Factors**

***Mary C. Ballentine**, Bonnie M. Ertel and John E. Weinstein, The Citadel*

Sources and pathways by which microplastics, plastic particles <5mm in size, enter coastal waters remains poorly understood. While studies have documented the presence of microplastics in urban and suburban atmospheric samples, there is currently a critical gap in our knowledge regarding the contribution of this pathway specifically in salt marsh habitats. The purpose of this study was to determine the contribution of atmospheric deposition of microplastics, including tire wear particles, into four salt marsh and salt marsh-adjacent sites around Charleston Harbor, SC (USA). To accomplish this, we collected samples using stainless steel buckets (324 $cm^2$) through passive deposition during both wet and dry periods. After a duration of between 1 and 7 days, retained particles were filtered onto glass fiber filters and visually analyzed under a dissecting microscope. Suspected microplastic particles were confirmed as synthetic with the hot needle test, and a subset were examined using FTIR spectroscopy. Preliminary results indicate that atmospheric deposition of microplastics at these four sites ranges from 88.2 to 3,458 microplastic particles/$m^2$/day. Microplastic abundance varied among our four sites, with the highest abundances associated with the salt marsh adjacent to a major bridge crossing Charleston Harbor (2,573 MP/$m^2$/day average ± 511.95 SE). Future research will investigate the morphological features of the particles (size and shape), including tire wear particles, and the correlation between particle deposition at these sites and meteorological factors (wind speed and direction, and precipitation). These results regarding atmospheric deposition of microplastics and tire wear particles will help us better understand the pathways and fate of these particles in our coastal waters.

**4.13.P-Mo119 Capture of Tire Road Wear Particles in Manufactured Treatment Devices, a Stormwater Pollution Best Management Practice**

***Kayli Paterson**[1], Barbara Beckingham[1] and John E. Weinstein[2], (1) College of Charleston, (2) The Citadel*

Tire road wear particles (TRWPs) emitted by vehicles due to the friction of tires on road surfaces are increasingly being recognized as a dominant fraction of microplastic/rubber in traffic-affected areas. TRWPs vary in density, size, shape, and composition. Due to high emissions and potential toxicity, preventing environmental dispersal of tire microrubber should be a priority. TRWPs are abundant in roadside soils and stormwater runoff and may accumulate in rain gardens and stormwater ponds. However, there is limited research on the fate of TRWPs in grey-stormwater infrastructure. We investigated TRWPs captured by advanced stormsewer basins called manufactured treatment devices (MTDs). Sediments were sampled from six MTDs in Mount Pleasant, South Carolina under dry conditions during scheduled clean-out. MTDs came in two varieties: a multi-chambered, flow-through baffle design and a single-chambered hydrodynamic vortex separator. Samples of road dust and sediment around the discharge site were also collected. Sediments were dried, sieved, and analyzed for grain size, organic matter, and heavy metal content. Density separations were performed on the target sieve fraction (63-500 micron) using a NaCl solution (1.2 g/mL) and then sodium polytungstate (1.9 g/mL) to distinguish low and high-density fractions. TRWPs in these fractions were identified by morphology and heat resistance, counted, and measured using stereomicroscopy. A subset of suspected TRWPs were taken for pyrolysis-GCMS and scanning electron microscopy for quality assurance. TRWPs varied in concentrations with density, roadway type, location, and sample type. Low density (<1.2g/mL) and high density (1.2-1.9 g/mL) TRWPs concentrations ranged from 50-2500 particles/g dry wt. and 25-9000 particles/g dry wt., respectively. The concentration of TRWPs in MTDs was around 2-3x higher than on roadways. While the relative abundance of low and high density TRWPs was similar between roadway and hydrodynamic vortex separator sediments, multi-baffled MTDs were more likely to capture high density TRWPs. The size of TRWPs decreased from roadway sources to MTD outfalls. Zinc and nickel, heavy metals associated with vehicles, strongly correlated to the abundance of TRWPs in sediments. Each MTD captured an estimated 0.4-1.9 billion TRWPs in sediments which were landfilled. MTDs are an available best management practices to reduce these problematic stormwater emissions.

**4.13.P-Mo121 Short-Term field measurement of Tire-Like particles as passive samplers for Polycyclic Aromatic Hydrocarbons in a South Texas Bay system**

***Nigel Lascelles**, Oluniyi O. Fadare and Jeremy L. Conkle, Texas A&M University, Corpus Christi*

Tire particles generated from tire abrasion during their everyday use on roads are major contributors to automobile traffic's non-exhaust emissions. With growing data on their ubiquitous environmental presence, we must understand their inherent and sorbed pollutant loads. This can be assessed with a passive sampler approach that deploys tire-like particles into environmental systems. The material deployed was determined by Pyrolysis Gas Chromatography Mass Spectrometry as Acrylonitrile butadiene styrene rubber polymer (ABS). The ABS was sieved to 500 mm before 7.5 g was added to a stainless-steel tea infuser and secured in a fish trap deployed at a dock in Port Bay, TX, for a four-week term. Duplicate samples were harvested at weekly intervals, extracted using accelerated solvent extraction, cleaned up using solid-phase extraction, and then analyzed for 16 polycyclic aromatic hydrocarbons (PAHs; US EPA 8720 mix) using Gas Chromatography with Triple-Quadrupole Mass Spectrometry. Research is ongoing, but this preliminary data will guide more comprehensive research to assess the role of tire particles in the transport and sorption of environmental pollution.

**4.13.P-Mo122 Chronic Effects of Microplastic and Microrubber Exposure on Grass Shrimp (Palaemon pugio)**

***Shannon Marie Bley**[1], John E. Weinstein[2], Peter B. Key[3] and Barbara Beckingham[1], (1) College of Charleston, (2) The Citadel, (3) National Oceanic and Atmospheric Administration (NOAA)*

Microplastic particles (MPs, <5mm) are prevalent in coastal environments. Tire wear particles (TWPs), a type of MP produced by tire abrasion on road surfaces, may present a higher risk than other MP types where surface waters are impacted by stormwater due to their abundance and toxic leachates. Several classes of estuarine organisms have been observed to ingest tire particles in the laboratory and field. Daggerblade grass shrimp (*Palaemon pugio*), an ecologically important and sensitive estuarine species found along the east coast of North America, is one such species observed to ingest and ventilate MPs and TWPs in laboratory studies. While acute toxicity due to MP and TWP exposure on this species has been documented, their chronic toxicity is currently unknown. This research aims to investigate the chronic effects experienced by grass shrimp due to long-term

MP and TWP exposure. Gravid grass shrimp will be collected from Leadenwah Creek, SC (USA) and acclimated to lab conditions. Once hatched, larval grass shrimp will be separated into beakers and exposed to one of four experimental conditions: pluff mud particles (control), crumb rubber as a proxy for TWPs, crumb rubber that has had latent metals and chemicals extracted, and a combination of MPs (fibers, fragments, foams, spheres) and crumb rubber reflecting the composition found in Charleston Harbor tidal creeks. Particles will range in size between 63-150 µm. Concentrations will be environmentally relevant, ranging from 0 to 250 mg/L, with the highest concentration reflecting those found in stormwater runoff. Shrimp will be exposed to particles for 24 hours every 5 days as a pulsed-dose exposure, simulating rain-induced runoff events in the Charleston Harbor area. Exposures will run for 10 weeks. Following exposures, shrimp will be frozen in liquid nitrogen (-80 °C) and stored until biomarker analysis. Assessed biomarkers include malondialdehyde (MDA)/thiobarbituric acid reactive substances (TBARS), glutathione, and DNA damage, with additional parameters including growth (in weight) and average number of molts to reach adulthood. The results obtained from this study will inform risk assessment of environmentally relevant MP and TWP concentrations for estuarine systems.

**4.13.P-Mo123 Comparative Toxicity of Micro and Nano-Scale Tire, Crumb Rubber and Recycled Rubber Particles to Zebrafish (Danio rerio).**

***Bryan J. Harper****, Brittany Cunningham, Miranda Jackson and Stacey L. Harper, Oregon State University*

Investigations into the occurrence of microplastics in the environment have resulted in the identification of numerous types of black rubber particles, many of which are attributed to tire wear on roadways. However; in addition to tire rubber being washed from roadways, crumb rubber, used in athletic fields, and recycled rubber, used for playground and park mulches, are also potential sources of black rubber entering the environment. We investigated the toxicological impacts of these various forms of black rubber to embryonic zebrafish in order to gain insights into the role of rubber composition and particle size. Although many studies have looked at the impacts of leachates, the current study looked at the effects of smaller cryomilled crumb and recycled rubber particles in the micro (1-20 µm) and nano-scale (<1 µm) size range on developing zebrafish embryos, relative to the impacts from tire tread particles we previously reported. Similar to our previously reported results for tire tread particles, both recycled and crumb rubber particles significantly delayed embryo hatching following nano-scale exposures; however, only micron crumb rubber particles resulted in hatching delays at concentrations >1 x $10^6$ particles/mL. Despite significant hatching delay, no significant mortality occurred following exposure to either size of crumb or recycled rubber at the highest test concentrations (1.3 x $10^6$ and 4.4 x $10^6$ particles/ml respectively for micron particles and 2.3 x $10^9$ and 2.8 x $10^9$ particles/ml, respectively for nanoparticles). These results suggest that exposure to black rubber, regardless of the source, may impact embryo hatching and development without impacting short-term survival.

**4.13.P-Mo124 Spatial Modelling of Airborne Microplastics Adsorption and Transport Characterisation on Heavy Metals in Coastal Environments**

***Precious Onyinyechi Odika*** *and Mike Fowler, University of Portsmouth, United Kingdom*

Microplastics (MPs) and heavy metals are ubiquitous polymeric contaminants and toxic chemicals respectively. They are classified as plastic particles with a size range ≤ 5mm in diameter. Microplastics and heavy metals contaminants have been found in many ecosystems on Earth, including air, water and soil, as well as the food chain of many species with potential health and environmental risks. Studies have shown extensively that microplastics serve as potential vectors of other contaminants especially in the marine, terrestrial and freshwater ecospheres. However, there exist few studies on atmospheric microplastics and their adsorption, desorption and transportation mechanism. This ongoing PhD study will identify airborne microplastics and examine how they influence the adsorption, release and transport of heavy metals of concern in the atmospheric environment of Portsmouth, UK and Nigeria in West Africa. We will investigate the chemical interactions between airborne microplastics and potentially toxic heavy metals through laboratory and field experiments. We plan to present comparative global simulations of heavy metal concentrations that are adsorbed and transported by microplastics in the atmospheric ecosystem. We will employ particulate fallout collector and ambient filter sampler for outdoor microplastics sampling at different sites. And further, subject the samples for laboratory extraction and identification of different types of microplastics. There will be evaluation of adsorbed heavy metals through SEM and ICP-MS analytical techniques. We will model and examine the mechanism and working conditions by which MPs adsorb, desorb and transport heavy metals and other toxic chemicals. Further discussion will be on the combined effects and exposure assessment of microplastics and heavy metals on the ecosystem species. The study will cover a range of analytical geochemistry and instrumentation development component that will contribute to internationally agreed protocols to airborne microplastics particles sampling, evaluation and monitoring procedures. A combined seasonal variation results will be used to simulate quantitative measurements on global air quality management and risk exposure assessment.

## 4.13.V In the Neighborhood and Out to Sea: Shedding Light on Tire Wear Microplastics

### 4.13.V-02 Investigating Tire Tread Particle Toxicity to Fish Using Rainbow Trout Cell Lines

***William Dudefoi***[1]*, Benoit J.D. Ferrari*[2]*, Florian Breider*[3]*, Thibault Masset*[3]*, Etienne Vermeirssen*[2]*, Alan J. Bergmann*[2] *and Kristin Schirmer*[1,3,4]*, (1) Eawag - Swiss Federal Institute of Aquatic Science and Technology, Switzerland, (2) Ecotox Centre CH, Switzerland, (3) Ecole Polytechnique Fédérale de Lausanne – EPFL, Switzerland, (4) ETHZ, Institute of Biogeochemistry and Pollutant Dynamics, Switzerland*

Tire and road wear particles (TRWP) are generated by the abrasion of tires while driving, and questions were raised recently about their potential contribution to microplastics released into the aquatic environment and their potential toxicological impacts. Our study aimed to assess the toxicity of TRWP and associated chemicals to fish using two Rainbow Trout (*Oncorhynchus mykiss*) cell lines representing the gill (RTgill-W1) and the intestinal (RTgutGC) epithelium. The toxicity of cryogenically milled tire tread (CMTT) particles, used as a proxy for TRWP, was assessed considering several potential exposure pathways: 1) exposure to CMTT with direct contact to quantify a combined particle/leachate effect, 2) exposure to CMTT leachate only to assess the toxicity of the leaching chemicals, 3) exposure to CMTT digestate to investigate if fish gastro-intestinal conditions could result in a different chemical profile and change the toxicity and 4) exposure to thermooxidised CMTT to determine the effect of aging. Following OECD TG249, cell viability was assessed after 24 hours acute exposure using a multiple-endpoint assay indicative of cell metabolic activity, membrane integrity and lysosome integrity. The exposure medium was analyzed to assess which chemicals could be responsible for the observed acute effects. The determined fish cell line *in vitro* EC50 values were 2.02 g/L and 4.65 g/L for RTgill-W1 and RTgutGC cell lines, respectively, in the same range as reported *in vivo*, and far above TRWP river water concentration (4 mg/L). Moreover, the presence of the particles greatly contributed to the overall toxicity, as the leachate alone induced lower toxicity. Aged CMTT also resulted in a lower toxicity, due to less chemicals leaching out in the medium. On the opposite, *in vitro* digestion of CMTT resulted in a higher toxicity in comparison to water leachates, with higher concentrations of metals and organic compounds leaching in the digestive fluids, such as Zn, 2-mercaptobenzothiazole, 1,3-Diphenylguanidine (DPG), and N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine (6PPD). Although traces of 6PPD-quinone were also measured, further cytotoxicity assays using RTgill-W1 and RTgutGC cell lines showed no toxicity up to 3 mg/L, while several studies reported toxicity *in vivo* at far lower concentrations. This could be due to a specific mode of action of this chemical, such as neurotoxicity, which we will investigate further using a Rainbow Trout brain cell line.

### 4.13.V-03 Searching for Safer Tire Anti-Degradants

***Craig Manahan****, Washington State Department of Ecology*

6PPD (*N*-(1,3-dimethylbutyl)-*N*′-phenyl-*p*-phenylenediamine) is an anti-ozonant used in tires to prevent cracking and premature aging. A transformation product of 6PPD, 6PPD-quinone, can form when 6PPD reacts with ozone. 6PPD-quinone is lethal towards coho salmon with a LC50 of <0.10 ppb, making it one of the most potent aquatic toxicants known. 6PPD-quinone is formed on tire particles and can be washed into streams and other bodies of water, where it will cause adverse affects on aquatic life.

This talk with overview the work that Washington Ecology is performing to help identify safer alternatives to 6PPD in automobile tires. Topics will include tools, methodology, and initial results. In addition, we will summarize other efforts underway at Ecology to address 6PPD contamination in Washington, including prioritization of areas for stormwater treatment, analytical method development, and environmental testing.

### 4.13.V-04 Chemical Composition of Road Pavement Particles and Tire Wear Particles - The Same or Not the Same?

***Stephan Wagner***[1]*, David Joshua Raithel*[2] *and Christopher Werner Funk*[2]*, (1) Hochschulen Fresenius Gemeinnützige, Germany, (2) Hof University of Applied Sciences, Germany*

Tire wear-, mineral-, and inorganic particles form heteroaggregates, the so-called tire and road wear particles (TRWP) at the road surface. Tire particles originate from tire tread which is abraded while driving. The mineral and inorganic components originate, for example, from road pavement abrasion or brake wear. Several studies have investigated the physical properties of TRWP, determining particle size distribution, particle shape and morphology. Only a few studies have addressed the chemical composition of TRWP. Such studies are needed to determine the chemical contribution of TRWP to road dust, and road sweepings, and to compare the chemical composition of TRWP generated under partially controlled conditions at tire-abrasion test sites with those TRWP taken from the real-world road environment. This data will also support the development of the representative test materials required for hazard assessment, for example, ecotoxicity tests.

In this study, we reviewed and systematically compared data from literature on the chemical composition of various tire wear particles of increasing complexity. We started with artificially generated tire tread particles (n=21), followed by TRWP from one tire abrasion test site (n=1), road dust from motorway tunnels (n=4) and road dust samples (n=64). The road dust sample from a tunnel was shown to have elevated concentration of tire wear particles compared to samples from road environment outside tunnels. In all investigated samples content of inorganic elements such as Cd, Co, Cr, Cu, Ni, Pb, and Zn were found and compared. All content were in the range of mg/kg, except for Zn, which was up to g/kg. The levels of Co, Cr, and Pb were clearly elevated in TRWP from one tire abrasion test site, while Cu and Zn ranged 15 times lower in artificially generated TRWP compared to road dust from a tunnel. Our study suggests that the composition of road dust may not fully be explained by TRWP and mineral particles from the pavement abrasion. It is possible that particulate sources such as brake abrasion contribute to the composition of TRWP in the environment. These comparisons of chemical composition suggest that further efforts are needed to develop and select appropriate test materials for TRWP, that would be, for example, better suited for ecotoxicity testing.

## 4.14 Innovative Analytical Approaches for Understanding Environmental Contaminants of Emerging Concern

### 4.14.T-01 Combining Analytical Techniques for the Measurement of Per- and Polyfluoroalkyl Substances (PFAS) in Plastic Products

***Heather D. Whitehead****, Megan Green and Graham F. Peaslee, University of Notre Dame*

Industrial processes account for the largest use of polymeric and non-polymeric PFAS worldwide and include the use of fluorinated polymer processing aids (PPAs) in plastic-product manufacturing. Since the 1980s fluorinated PPAs have been used to increase the speed and lower the cost of manufacturing across a broad range of end products including food packaging, shopping bags, and liquid bottling. Niche applications, including the manufacturing of artificial turf grass, have also been reported to use fluorinated PPAs. The extent to which both the manufacturing and use of fluorinated PPAs might contribute to PFAS measured in the products that use them is currently unknown. To assess this environmental threat, a combination of analytical techniques including targeted LC-MS/MS, total fluorine, extractable organic fluorine, and Total Oxidizable Precursor (TOP) assay were utilized to screen for PFAS in thin-film and molded plastics in 49 samples of artificial turf, product packaging, and food packaging. Total fluorine measurements from particle-induced gamma-ray emission (PIGE) spectroscopy produced concentrations ranging from non-detect to 480 parts-per-million of fluorine. Following total fluorine screening samples were subjected to QuEChERS extraction and analyzed with LC-MS/MS. Targeted analysis of 21 PFCAs and PFSAs yielded "sum of targeted PFAS" concentrations that ranged from <LoD - 10 ng/g material (median of 1 ng/g) with PFHxS, PFBS, and PFPeS having the highest detection frequencies at 16%, 16%, and 13% respectively. A subset of sample extracts was analyzed using PIGE to measure extractable organic fluorine before being subjected to the TOP assay to determine precursor PFAS content. Results from these plastic products were compared to results generated from two raw fluorinated PPAs to better understand the relationship between PFAS content in both the raw material and final products. These preliminary investigations to identify total PFAS present in plastics are critical for researchers and regulatory bodies alike when determining the potential for environmental and human exposure from continued use of fluorinated PPAs in consumer goods and products.

**4.14.T-02 Analysis of Plastic Additive Leaching Kinetics Using High Performance Liquid Chromatography with Quantitative Time-of-Flight Mass Spectrometry**

***Eric Fries*** *and Roxana Suehring, Ryerson University, Canada*

Plastic additives are a diverse group of chemical compounds added to plastic products to give them their unique physical-chemical properties. Plastic additives can leach out of products and into the aquatic environment, posing a potential ecotoxicological risk. In addition, some commonly used plastic additives can be classified as persistent, mobile, and toxic (PMT). These highly polar, environmentally stable contaminants can pose a significant, long-term risk to the aquatic environment. While some studies have assessed the kinetics of plastic additive leaching, many of those investigated a small group of additives or additive classes, focused on short time frames, or used leaching conditions that are not environmentally relevant. There is a need to assess the long-term kinetics of PMT plastic additive leaching in different plastics within environmentally relevant matrices and weathering conditions. The presented study investigated the leaching kinetics of three plastics (crumb rubber, polypropylene, polyvinyl chloride) commonly detected in the environment using artificial weathering experiments that altered water type (lake water, artificial sea water), pH, and UV-light exposure over a 60-day period. PMT plastic additives were measured using high performance liquid chromatography with quantitative time-of-flight mass spectrometry (HPLC-QToF-MS). A range of PMT plastic additive suspects were detected in the leachate samples including benzothiazole, N,N-diphenylguanidine, tetramethyl decynediol, and organophosphate esters such as tris(2-butoxyethyl) phosphate and triethyl phosphate. In general, the levels of PMTs in the leachates plateaued over the 60-day period, with some exceptions. UV-light exposure enhanced the leaching of some PMTs, as did lower pH (6.0). Additionally, water type influenced the leaching of some PMTs, but ultimately, leaching kinetics were generally compound- and polymer-specific. The results from this study provide a better understanding of the presence of PMT plastic additives in plastics commonly found in the environment, the general leachability of these PMTs, and what environments and weathering conditions may enhance their leaching. This can provide future works with an effective, environmentally-relevant leaching and analysis protocol to assess the environmental contamination with PMT plastic additives from a variety of plastic products.

**4.14.T-03 Novel Approaches to Elucidate Self-assembly Behaviors of a Cationic Polyfluoroalkyl Substance on Clays**

***Bei Yan***[1]*, Jinxia Liu*[1]*, Yanjie Shen*[2] *and Xiao-Ying Yu*[3]*, (1) McGill University, Canada, (2) Ocean University of China, (3) Oak Ridge National Laboratory*

Fluorosurfactants used in aqueous film-forming foams belong to the large chemical class of per- and polyfluoroalkyl substances (PFAS), many of which are highly persistent, bioaccumulative and pose negative ecological and human health effects. These surfactants are water soluble, so their strong retention by solid surfaces cannot be adequately explained by established sorption paradigms. The scientific challenge calls for new approaches to study the surface retention of PFAS and improve mechanistic understandings. We hypothesize that fluorosurfactants can spontaneously aggregate at water-soil (e.g., clay) interface to form "self-assembly", contributing to their excessive retention. Time-of-flight secondary ion mass spectrometry (ToF-SIMS), a surface-sensitive mass spectral imaging tool was employed to probe the self-assembly structure of a cationic surfactant at the water-clay interface and in water, respectively. The ToF-SIMS depth profile implied a multilayer structure formed by the cationic compound, and its arrangement on the clay surface followed the (negative) charge distribution as a result of the Al-Mg iso-substitution. The all-atom molecular dynamics simulation, offering a "seeing is believing" way, visualized the diverse self-assembly structures at the atomic level. The detailed and accurate picture of how PFAS interacts with the clay was investigated by the density functional theory (DFT) quantum calculations, which determines atomic forces and system energy from the first principles. The DFT-based thermochemistry results indicated that the spontaneous micellization with elevating concentration/adsorption density was entropy-driven and dominated by the hydrophobic effect. The diverse self-assembly structures were primarily stabilized by the dipole-dipole (CF chain-CF chain and CH chain-CF chain) and charge-dipole (charge-CF chain) interactions arising from the excess electron density on the fluorine atom. The electrostatic attraction between the quaternary ammonium group of cationic PFAS and the polyhedral cavity formed by 6 corner sharing $\{SiO_4\}$ tetrahedrons of clay played a critical role at the stage of sparse occupation and monolayer. The complementary use of these computation tools predicted the mutual influences between PFAS and surface chemistry, clay aggregation and transport of PFAS, showing great agreements among ToF-SIMS and other tools (e.g., $^{19}$F NMR, and SEM).

**4.14.T-04 Understanding Fate and Distribution of Antimicrobial Compounds and Antimicrobial Resistance Genes in Wastewater Treatment**

***Charlotte Head*** *and Jonathan Brett Sallach, University of York, United Kingdom*

Antimicrobial resistance (AMR) is a global health crisis, rendering pharmaceutical action against infections ineffective, threatening lives worldwide. The AMR challenge is complex as both exposure to antimicrobials as well as the transfer of antimicrobial resistance genes (ARGs) can result in the proliferation of AMR. Wastewater treatment plants (WWTPs) are hotspots of AMR and reservoirs for antimicrobial compounds and ARGs. Both are considered contaminants of emerging concern. As WWTPs receive the unmetabolized portion of antimicrobials excreted by a population, the mixture of antimicrobial compounds and subsequent ARGs is very complex. In order to gain new insights into the exposure of and interaction between antimicrobials and ARGs, innovative analytical approaches must be developed.

In this study, samples were collected throughout the treatment process for microbial and chemical analysis from a WWTP in York, England. SmartChip Real-Time PCR was employed to quantitatively monitor 208 relevant ARGs and related genes. Chemical screening using High-Resolution Mass Spectrometry (HRMS) was performed using Thermo Scientific Fusion Orbitrap mass spectrometry with analysis in a custom workflow developed within Thermo Scientific Compound Discoverer software.

SmartChip PCR revealed the presence of 119 of the 208 genes investigated, indicating the conference of resistance to multiple major antimicrobial classes including beta-lactams, sulfonamides and tetracyclines. The non-target screening of wastewater samples tentatively identified more than 20 antimicrobial parent compounds as well as biologically active metabolites and transformation products. Data visualization conducted in R (version 4.1.3) helped to reveal patterns in the levels of ARGs throughout the treatment process as well as connecting the presence of ARGs with detected antimicrobials. These results provide new insights into the dynamics of AMR within a critical hotspot in wastewater treatment.

**4.14.T-05 The Permeability of RTgill-W1 Cell Membranes to Per- and Polyfluoroalkyl Substances: A Case Study for Linking Toxicokinetics with Toxicodynamics**

***Ross M Warner***[1]*, Anne M. Mayo*[2]*, Keri B. Donohue*[2]*, Ashley N. Kimble*[2]*, Timothy C. Schutt*[2]*, Brian D. Etz*[1] *and Michael L. Mayo*[2]*, (1) Oak Ridge Institute for Science and Education (ORISE), (2) U.S. Army Engineer Research and Development Center*
Per- and polyfluoroalkyl substances (PFAS) are persistent contaminants of concern that pose substantial challenges for effective risk and hazard assessments. In a previous work, we proposed a novel toxicokinetic model for PFAS in the early development zebrafish (*Danio rerio*) by incorporating physiological dynamics throughout embryogenesis. As a result, the model is potentially predictive at longer timescales than other models and could be applied to other chemical classes. Ultimately, we propose to leverage this toxicokinetic model to inform zebrafish toxicodynamics via novel quantitative adverse outcome pathway (qAOP) models. These and other models will help to integrate the growing field of qAOPs with risk assessment by enabling the toxicological interpretation of environmental (fish in particular) and human health data. To support this goal, we refined tissue-level PFAS concentrations, predicted by our toxicokinetic model, into extracellular and intracellular components. This distinction is important, because the molecular initiating events of AOPs can occur in localized regions. To address this concern, we sought to resolve timescales associated with PFAS transport across lipid membranes of gill cells via a transwell assay, in which we measured PFAS concentrations via LC-MS/MS over time. Using these data, we developed a mathematical model to predict PFAS permeabilities, which displayed good agreement with literature values identified for other cell types. These permeability estimates indicate that PFAS transport occurs on the order of minutes for a single cell within a zebrafish embryo. Considering that PFAS transport at the level of embryo tissues occurs over a timescale of hours to days, we deduced a linear relationship between the extracellular and intracellular concentrations. In contrast, we will present progress on a purely in silico approach we developed to estimate PFAS partitioning in the zebrafish embryo, which is relevant for rapid hazard screening of PFAS. We describe a novel approach to calculate PFAS permeability across a lipid bilayer model that depends only upon the potential of mean force, the viscosity of the lipid bilayer, and the extent of thermal noise—all of which can be calculated from a series of molecular dynamics simulations. We compare these permeability values with those obtained from before, to better discriminate the role and relevance of transporter proteins to zebrafish embryo toxicology.

**4.14.T-06 Passive Sampler for the Time-Integrative Measurement of Per- and Polyfluoroalkyl Substances in Water**
***Paul Edmiston***[1]*, Riley Hershberger*[1]*, Craig Divine*[2] *and Erika Carter*[2]*, (1) College of Wooster, (2) Arcadis U.S., Inc.*
Per- and polyfluoroalkyl substances (PFAS) are a class of persistent contaminants routinely monitored in the hydrosphere. Passive sampling for environmental monitoring has the advantages of reducing sampling costs and providing the time-averaged concentration for the duration of the sampling period in diverse environmental matrixes. Here, a 2.5 cm x 4.5 cm x 0.2 cm polyethylene device was developed that houses a PFAS-specific adsorbent behind open mesh. Adsorbent resin was synthesized from mesoporous hydrophobic organosilica modified with polyethylenimine (PEI) and Cu(II) ions to add cationic adsorption sites. Passive samplers were characterized in bench-scale measurements varying flow rate, water chemistry, and PFAS concentrations. Field trials deploying samplers in either surface water or groundwater were conducted at Ellsworth and Peterson Air Force Bases, respectively. Key findings included: i) an integrative response over time periods of at least 3 months; ii) the ability to incorporate surrogates into the analysis for isotopic dilution protocols; and iii) good agreement between grab and passive sampler water results across >5 orders of magnitude. The presentation will provide details on the sampler design, deployment options, and measurement method. Finally, the application of samplers to measure PFAS in stormwater and sediment pore-water matrixes will be described.

**4.14.T-07 Integrated monitoring of emerging pollutants in the aquatic environment: the combined use of passive sampling and electrochemical sensing technique.**
***Pheladi Lizzy Mokaba****, Usisipho Feleni, Titus A.M. Msagati and Hlengilizwe Nyoni, University of South Africa*
One of the major challenges in most monitoring programmes is that many of the toxic compounds present in

water exist in very low concentration thus presenting a significant challenge in their measurements. This has forced the world to embark on developing new techniques with lower detection limits in order to inform the correct selection of treatment technologies that can completely remove or degrade such contaminants to harmless products. In the last decade, advances in technology and data analysis tools have seen the success of integrating different types of techniques and methods in order to validate the data obtained in different ways. However, the combined use of passive sampling and sensing technologies for water quality monitoring is yet to be explored. In this work, the potential of integrating electrochemical sensing and passive sampling methodologies was investigated. Passive sampling methodologies are cost-effective tools useful for measuring ultra-trace micropollutants in water. Conversely, electrochemical sensing devices are tools capable of giving information about the composition of a system in real time. Robust passive sampling techniques were tested, and an electrochemical sensor developed for the detection of a wide range emerging contaminants including PFOS and PFOA. Ultimately, a multidimensional real-time water monitoring system was built. The necessary infrastructure was configured to allow placement in water while providing automated data transmission and processing via the wireless monitoring system. The potential use of the integrated monitoring system was evaluated in a domestic wastewater treatment works facility.

**4.14.T-08 Poster Highlights: Innovative Analytical Approaches for Understanding Environmental Contaminants of Emerging Concern**

***Theresa Guillette**, Arcadis U.S., Inc.*

In this poster highlights time slot, the authors of some of the excellent posters submitted to the session will provide an overview slide presentation to highlight the major findings in their poster presentation.

**4.14.P Innovative Analytical Approaches for Understanding Environmental Contaminants of Emerging Concern**

**4.14.P-Tu104 Upcoming Environmental Reference Materials for Per- and polyfluoroalkyl substances (PFAS)**

***Jessica Lynn Reiner**, Alix E. Rodowa and Benjamin Place, National Institute of Standards and Technology (NIST)*

Standard Reference Materials (SRMs) are homogeneous, well-characterized materials that are used to validate measurements and improve the quality of analytical data (www.nist.gov/srm). The National Institute of Standards and Technology (NIST) has a wide range of SRMs that have values assigned for legacy organic pollutants. These SRMs can serve as target materials for method development and quality assurance and quality control (QA/QC) of chemical measurements. The general overview for the use of reference material for environmental measurements will be discussed. As a unique class of organic contaminants, per and polyfluoroalkyl substances (PFAS) present measurement challenges to the environmental analytical community that can affect the accuracy and precision of quantitative measurements. Currently, NIST has eleven different reference materials with values of PFAS measured in them; however, there are relevant gaps in the NIST library of reference materials. A major gap is a reference material of high level PFAS, including technical mixtures, specifically an aqueous film-forming foam (AFFF) material, and contaminated soils. This presentation will discuss the existing PFAS environmental reference materials, along with the past and current efforts at NIST to produce new reference materials for PFAS in environmentally relevant matrices.

**4.14.P-Tu105 Assessing the Impacts of Sub-Chronic Exposure to Emerging Pollutants on Activity and Species Variability in a Marine Amphipod**

***Bidemi Green-Ojo**[1], Hung Tan[2], Lena Grinsted[1], Matthew Parker[1] and Alex Ford[1], (1) University of Portsmouth, United Kingdom, (2) Monash University, Australia*

Plasticizers are commonly used to increase the flexibility and durability of plastics across a wide variety of personal and household products. There is growing evidence that emerging pollutants such as nbutylbenzenesulfonamide (NBBS) and Triphenyl phosphate (TPhP) are increasingly studied as pollutants of emerging concern due to their ability to cause neurotoxic and endocrine disruption effects in humans. However, little is known about how these plasticizers can alter the behavioural traits of amphipods. This study evaluates the impact of sub-chronic exposure of NBBS and TPhP on swimming behaviour and analyzes variability among and within species of a marine amphipod, *Echinogammarus marinus*. Animals were exposed to environmentally relevant concentrations of NBBS and TPhP for 14 days and the nominal concentration was analyzed to verify the actual concentration exposed to the animals. Mortality and moulting were recorded daily. The swimming behaviour was tracked on day 7 and day 14 using DanioVision and the EthosVision video tracking software to record the exposed animals' velocity, distance, and activity state. The presentation will discuss the extent to which plasticizers can influence the behaviour of amphipods, provide evidence of variability within and between species, and confirm the repeatability of the study.

**4.14.P-Tu106 Evaluation of the Development of Health-Based Drinking Water Guidance Values for PFOA and PFOS in Regulatory Jurisdictions Around the World**

***Francis Ramacciotti****, Ian Collins and William A. Schew, GHD*

Environmental contamination of drinking water by per- and polyfluorinated alkyl substances (PFAS) has become a widespread and widely-recognized health and regulatory concern. Two PFAS in particular, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), have come under review due to their widespread use, documented water impacts, and persistence in the environment. A number of regulatory agencies across the world have developed their own toxicity criteria and derived drinking water values, demonstrating a lack of regulatory consensus in selecting PFAS toxicity criteria. In the interest of providing clarity for stakeholders who are looking to manage sites with PFAS concerns, this paper evaluates the assumptions and approaches used by regulatory agencies in the United States, Canada, Europe, and Australia and New Zealand that have developed PFAS criteria. We hope that this information can be useful to not only site managers but also regulators who may be looking to set PFAS values in the future. The objectives of the study were as follows:

(a) Examine approaches and assumptions used to derive Toxicological Reference Values (TRVs) and Health-Based Guidance Values (HBGVs) for PFOS in drinking water and PFOA across the world.

(b) Provide clarification and useful advice to stakeholders concerning best-supported information and context for decision-making.

Through our evaluation, we found that evidence supports specific toxicokinetic models, critical organ systems, UFs, and RSCs to varying degrees. For both compounds, the use of the Wambaugh et al. (2013) toxicokinetic (TK) model appeared to be well-supported. The selected Human Equivalent Dose (HED) calculation methods, however, directly influenced the chosen critical organ system for both compounds. This influence was more evident in PFOS target endpoints than for PFOA. The review considered that UFs should be influenced by the principal study chosen. We recognized that some UFs could be study specific, while others could be applied based on the body of evidence. We also found evidence to support modifications to currently utilized UFs. Finally, the selection of Relative Source Contributions (RSCs) by agencies were influenced by exposure models, epidemiological data, and the agencies' own TRVs. RSCs above the EPA default were supported in the review.

**4.14.P-Tu108 Evaluation of Tissue Extraction Methods for Per/Polyfluoroalkyl Substances (PFASs) in Benthic Marine Biota**

***David Dukes*** *and Carrie A. McDonough, Stony Brook University*

Per/polyfluoroalkyl substances (PFASs) have impacted water resources across the United States due to their widespread use in aqueous film forming foam (AFFF) used to fight liquid fuel fires. AFFF releases at coastal sites have led to PFAS contamination of marine sediments and surface waters. Sediment-associated PFASs are taken up by benthic biota, thereby entering marine food webs. Evaluating bioaccumulation and biomagnification of PFASs in AFFF-impacted marine environments requires accurate, repeatable, and efficient tissue extraction methods. Many different methods are used in the literature, making it difficult to choose the optimal method for a given study. Choice of an optimal method likely depends on the resolution and sensitivity of the analytical instrument to be used and the degree of contamination in the samples of interest. Here we conducted a study to evaluate several approaches for extracting PFASs from tissue of marine biota. Initial comparisons were completed using tissues from marine worms (*Alitta succinea*) exposed to eight PFASs for seven days. Method performance was evaluated using tissue from depurated worms fortified with structurally diverse PFASs including C3-C14 perfluorocarboxylic acids (PFCAs) and C4-C10 perfluorosulfonic acids (PFSAs). Methods considered in the comparison include an acidified methanol protein crash and serial sonication with basic methanol. EPA Draft Method 1633 was also adapted for smaller tissue amounts and evaluated. Preliminary results suggest that two serial basic methanol extractions with sonication followed by analysis on a high-resolution quadrupole time-of-flight mass spectrometer (QTOF-MS) provided consistent recoveries (between 70-130%) for a wide range of PFASs.

**4.14.P-Tu109 Development of a Semi-quantitative Non-Targeted Analysis Workflow for Emerging PFAS**

***Shirley Pu*[1]*, James McCord*[2] *and Jon Sobus*[2]*, (1) Oak Ridge Institute for Science and Education (ORISE) participant at U.S. Environmental Protection Agency, (2) U.S. Environmental Protection Agency***

Emerging chemical contaminants present a challenge for traditional chemical quantification as they lack reference standards; this is particularly true for per- and polyfluoroalkyl substances (PFAS) which have a wide range of structural diversity but limited availability of standard reference compounds. In the absence of available standards to estimate chemical concentrations for emerging compounds detected via non-targeted analysis (NTA), surrogate calibration curves based on related chemicals can be used to obtain semi-quantitative concentration estimates. The uncertainty of the concentration estimates is influenced by error inherent to NTA workflows, the choice of the surrogate(s), and the mathematical model used to predict the concentration from the observed instrument values. Obtaining accurate and statistically bounded estimates of chemical concentration using NTA data is an important step for interpreting environmental measurements in a risk-based context. To address these gaps, we first compared the performance of 4 different mathematical models using a semi-quantitative non-targeted analysis workflow that provides concentration estimates with confidence intervals. The 4 models were initially assessed using a set of 16 legacy PFAS compounds with matched reference standards. We additionally compared the estimation error and uncertainty when using data processed using targeted and non-targeted workflows. Estimation error and uncertainty were larger for non-targeted analysis data, but performance of a given model type was generally similar regardless of the data type. However, calibration curves created using one data type did not predict accurate concentrations for the other data type. Based on the model comparison, the choice of workflow model was finalized as the linear log-log calibration curve. Semi-quantitative concentration estimates were then obtained for a set of emerging compounds, using the 16 PFAS reference standards to construct surrogate calibration curves for each compound. The estimation error and uncertainty were compared across surrogates to select the best surrogate for each compound. In the future, the developed NTA workflow may be used to expand analysis of emerging compounds from relative concentrations to estimated concentrations that can contribute to provisional risk evaluation.

**4.14.P-Tu111 Laboratory Determination of Per- and Polyfluoroalkyl Substance Sorbent-Water Partitioning: Best Practices.**

***Jarod Snook****, Jitka Becanova and Rainer Lohmann, University of Rhode Island*

The characterization of sorbent affinity for a target compound (described by sorbent-water partitioning coefficient, $K_{sw}$) is a necessary step in the sorbent selection and performance-testing process in passive sampling and remediation fields. However, no standardized procedure exists to measure $K_{sw}$, and the few available studies vary significantly. For per- and polyfluoroalkyl substances (PFAS), most $K_{sw}$ determinations employ batch experiments with small-scale water-sorbent mixtures, methanol-based spike of target compound(s), and analysis after assumed equilibrium, but quantities and methodological details of the above procedure differ. Therefore, we conducted several batch experiments systematically varying a general partition coefficient procedure to characterize the effects of sub-optimal experimental design. Using a subset of PFAS compounds (each with 6-carbon fluorinated chain length with differing functional groups) and two sorbents, we tested variations of solution:sorbent ratio, methanol content, and PFAS initial concentration and compared resulting $K_{sw}$ values. Each methodological component affected $K_{sw}$, usually by suppressing the value when compared with an optimized procedure. Thus, we suggest (1) an optimized procedure for target PFAS and sorbents used in this study, and (2) general guidelines for partitioning batch experiment design with different compounds and sorbents. Additionally, we report well-constrained $K_{sw}$ values for the four PFAS and two sorbents used here—an important parameter in passive sampling and remediation of PFAS in the environment.

**4.14.P-Tu112 Validation of a High-Resolution Passive Profiler (HRPP) for PFAS via Flow Box and Traditional Sorption Studies**

***Morgan Eldridge**, Kaylin McDermett, Jessica LaFond, Todd Anderson and William Jackson, Texas Tech University*

Characterizing per- and poly-fluoroalkyl substances (PFAS) in diverse hydrogeologic conditions is a major undertaking for the collection of site-specific data. To aid in this effort, a passive profiler has been designed that is capable of providing data to estimate sediment pore water velocity, hydrogeological and geochemical parameters, as well as characterizing subsurface contaminants in high resolution (~ 20 cm). The ability of this HRPP model to provide the data expected was verified via deployment in a large flow box filled with kaolin clay and topped with coarse sand, saturated with 15 PFAS in water. This deployment was done in duplicate: one HRPP deployed for three weeks and one HRPP for four weeks. The profilers were subsequently removed and sampled immediately to calculate the extent of equilibrium established (est. > 90% at three weeks), porewater velocity, and PFAS contaminant profiles quantified using ion chromatography (IC) and high-performance liquid chromatography-mass spectroscopy (HPLC-MS). Due to the potential strong sorptive properties of some PFAS, we conducted an additional study to quantify PFAS sorption to sampler materials to ensure the material makeup of the HRPPs was compatible with PFAS analysis. Each of the materials used to construct HRPPs was subjected to a traditional sorption study to determine the sorption capacity and ensure that the required time to reach equilibrium would not be extended due to surface interactions of PFAS. Materials tested included nylon membrane, stainless steel membrane, Viton™ gasket, and two thicknesses of high-density polyethylene (HDPE) gasket. Squares of each material (roughly 1" x 1") were placed in various concentrations of the same 15 PFAS mixture in water and sampled once a week for 28 days. The resulting data were used to calculate the sorption equilibrium amount ($q_e$) of PFAS to the materials to ensure they will not hinder equilibration when used in the field. Together, these studies will help determine if the HRPP design has been optimized for successful future use in the field.

**4.14.P-Tu113 The Development of a Diffusion-Based Equilibrium Passive Sampler for PFAS Detection and Exposure Assessment in Sediment Pore Water and Surface Water**

*Brent Pautler[1], Alex Sweett[1], Iryna Ilina[1], Jeff Roberts[1], **Michael Healey**[1], Anh Pham[2], Florent Risacher[3], Blessing Medon[2], Rachel Zajac-Fay[3], Lisa D'Agostino[3], Jason M. Conder[3], Jeremy Gauthier[4], Scott Mabury[4], Amila O. De Silva[5], Andrew Patterson[6], Patricia McIsaac[6] and Robert Mitzel[6], (1) SiREM, Canada, (2) University of Waterloo, Canada, (3) Geosyntec Consultants, Inc., (4) University of Toronto, Canada, (5) Environment and Climate Change Canada, (6) Eurofins Environment Testing (EET)*

Per-and Polyfluoroalkyl Substances (PFAS) have emerged as a concern in the environment due to their persistence, bioaccumulation in living organisms, and toxicity. The established sampling protocols and PFAS concentration determination in sediment and surface water currently only captures the total concentration at a single timepoint and represents the entire mass of PFAS present, which may result in an overestimation of the bioavailable PFAS exposure to human and ecological receptors. Equilibrium passive sampling is a popular approach used to assess bioavailability and risk through the dissolved phase of contaminants but as PFAS are emerging contaminants, researchers have only started investigating potential passive sampling solutions. Given their partial water-solubility and the ability of analytical laboratories to detect trace amounts of PFAS in water, a diffusion-based equilibrium passive sampler was hypothesized to be a good passive sampling device candidate. When deployed, analytes dissolved in the water or sediment equilibrate with the water in the sampler through the semi-permeable membrane. Through a series of bench-scale laboratory experiments, factors affecting the migration of several carboxylate and sulfonate PFAS into a diffusion cell were tested including the type of membrane filter, filter size, and solution chemistry. The results of these experiments suggested that the uptake of PFAS into the sampler was the fastest with polycarbonate membrane-based samplers, that the solution chemistry did not influence the PFAS uptake, PFAS were not lost to sorption or was not produced from sampler materials and that this sampler could be used to monitor multiple PFAS compounds.

The laboratory validated sampler was further tested in an *in-situ* field pilot to measure PFAS in sediment porewater and surface water. Targeted analytical results (Modified EPA 537, EPA 1633) were successful and suggested that equilibrium was reached in 14 days for surface water, an average 75% equilibrium for all target compounds detected in porewater after 28 days and were all within a factor of 2 or less with averaged grab sample results. Non-targeted analysis from the samplers showed more diversity in species and the applicability for detection of additional PFAS analytes with this sampler. Future experiments include direct comparison with tissue samples to further validate the relationship between passive sampling results and exposure, risk and bioaccumulation.

**4.14.P-Tu114 Recent Advances in Automated Sample Preparation using Accelerated Solvent Extraction**
***Chris Shevlin**, Rahmat Ullah, Changling Qiu, Anzi Wang, Husam Al-Esawi, Mingfang Wang and Fabrizio Galbiati, Thermo Fisher Scientific*
Accelerated solvent extraction is a high-temperature and high-pressure extraction technique that is widely used in environmental, chemical and food analysis. Extractions at high temperatures and pressures allow faster extraction of analytes relative to conventional solid-liquid or liquid-liquid based extraction techniques. Moreover, parallel extraction offer the advantage of faster operation. Gas assisted extraction is a method of extracting an analyte from a sample that uses a mixture of a liquid solvent and a gas through the sample container. Gas assisted extraction allows the extraction to proceed at low flow of solvent continuously.

In this presentation, we discuss a new parallel extraction protocol for pursuing extractions in gas assisted extraction mode followed by quantitation for persistence organic pollutants (POP). We present here performance data of the new method for analyte extraction and evaporation in the same platform from soil samples for different POP. Unlike traditional methods such as liquid-solvent extraction followed by N2 stream evaporation, the fully automated solvent extraction and evaporation system saves time, solvent, and labor, while ensuring high reproducibility and productivity for analytical testing.

**4.14.P-Tu115 Evaluating Subsurface Movement of PFAS Compounds Using Both One and Multi-Dimensional Modeling Approaches**
*Colleen Roy, **Brenna Kent**, Gerco Hoogeweg, Amy Ritter and Raghu Vamshi, Waterborne Environmental, Inc.*
Per- and polyfluoroalkyl substances (PFAS) are used in in numerous consumer products and industrial applications. Not only are PFAS widely used, but they also cover a vast chemical group with thousands of distinct compounds. Because of this, they have become a ubiquitous occurrence in the environment. Several small and large-scale monitoring programs have shown widespread presence of these compounds in air, surface and ground water, and soil media. As awareness of these chemicals continues to increase, concerns due to their persistence and toxicity to environmental and human health also grows. Through their PFAS Strategic Roadmap, USEPA has been prioritizing ongoing work to better understand and eventually reduce the potential

exposure and risks caused by these chemicals. USEPA has also included 29 PFAS to be monitored under the fifth Unregulated Contaminant Monitory Rule (UCMR 5) between 2023 and 2025. In addition to monitoring data, reliable modeling tools to evaluate the fate and transport of these chemicals are critical to the development of risk assessment and remediation strategies. To date, limited work has been done to better understand the fate and transport of these complex chemicals in the environment using existing modeling approaches. Rising public interest and increasing regulatory action has made the need for modeling an important next step in advancing the understanding of these persistent chemicals. This work will focus on applying standard modeling approaches to understand the fate and transport of PFAS. Two models, HYDRUS and GeoPEARL, were used to simulate PFAS measured at contaminated sites. Previously, these models were applied to simulate PFOA and PFOS in groundwater at an airport which had been used as firefighter training site, exposing the area to AFFF. This work has been expanded to include sites with other potential PFAS sources such as landfills. Results from modeling were compared with available groundwater monitoring data for these sites. The practical utility of the standard modeling approaches for application to address the PFAS challenges over small and large geographies are discussed.

**4.14.P-Tu116 Detection and Quantification of Brominated Natural Products in Arctic and Mid-Latitude Coastal Air and Waters**

***Emma Shipley***[1]*, Penny Vlahos*[1] *and Terry F. Bidleman*[2]*, (1) University of Connecticut, (2) University of Umeå, Sweden*

Halogenated natural products are organic compounds produced by marine bacteria and other marine organisms, as well as created anthropogenically through water chlorination and production as industrial compounds. These compounds can exhibit toxicity, bioaccumulate in the environment, and perform important roles in the regulation of the tropospheric and stratospheric ozone. To date, these compounds are understudied, particularly in Arctic and mid-latitude systems, with limited global data or understanding of spread. This project will share data on both di- and tri- bromoanisoles and bromophenols from two contrasting coastal zones including the Western Arctic collected on a May-June 2021 cruise in the Beaufort and Chukchi Seas, as well as data collected from the mid-latitude estuary of Long Island Sound during 2022. These efforts help to close the gap in measurements of HNPs and inform future studies.

**4.14.P-Tu117 Effect of Organic Matter Functional Groups on Per- and Polyfluoroalkyl Substances (PFAS) Adsorption**

***Yaseen Al-Qaraghuli*** *and Erica R. McKenzie, Temple University,*

Per- and polyfluoroalkyl substances (PFAS) have been ubiquitously found in groundwater, soil, and many other environmental matrices. Previous work has revealed that soil-associated PFAS are often predominantly cationic and zwitterionic compounds, while anionic PFAS comparatively are more in the dissolved phase. For many contaminants, sorption to soil is often driven by interactions with organic matter; for PFAS, mixed effects have been observed for sorption to soil organic matter. Organic matter functional groups, which are generally not evaluated, may also affect sorption capacity. This study has been initiated to study these themes and significant progress will commence in July. In the planned study, the impact of organic matter functional groups and charge will be assessed using a variety of PFAS compounds, including zwitterionic, cationic, and anionic PFAS. Polymers will be used to mimic the functional groups and the organic matter hydrophobic components and polypropylene beads will be used as a soil proxy. Carboxyls and phenols are major acidic functional groups in organic matter and dissociate at pH > 5. The major basic functional groups are amine and amide that dissociate at higher pH (>9). For organic matter proxies, poly(acrylic acid) and phenolic formaldehyde that are expected to be negatively charged at environmental pH values while poly(allylamine hydrochloride) and polyacrylamide are expected to be positively charged at environmentally relevant pH values. Batch experiments will include environmentally relevant concentrations of PFAS with polymer-coated polypropylene beads. The batch reactors will be operated at a range of pH values – 2, 4, 6, 8, 10, and 12 – to study the effect of dissociation status of PFAS and the polymers on their interactions. It is hypothesized that zwitterionic and cationic PFAS will be mainly sorbed onto negative charged polymers and anionic PFAS will be mainly sorbed onto positive charged polymers. Understanding the impact of the organic matter functional groups on the sorption capacity for PFAS is crucial to better understand compound-specific organic carbon-water distribution coefficients (log $K_{oc}$); results from this study will be used to develop sorption linear free energy relationships (LFERs).

**4.14.P-Tu118 Per- and Polyfluorinated Alkyl Compounds (PFAS) Analysis of Textiles and Food Contact Paper Products**

***Craig M. Butt*** *and Mikyanny Reyes, SCIEX*

Per- and polyfluorinated alkyl compounds (PFAS) are added to textiles and food contact papers for their stain-repellency properties. These applied PFAS compounds can migrate from the textiles and paper products, ultimately contributing to human exposure. Therefore, there is a need for highly sensitive and robust analytical methods to measure PFAS in consumer products. In this study we combined a simple extraction procedure with the high sensitivity of the SCIEX 7500 system for the quantification of PFAS in a broad range of commercially available products. All products were advertised as having either stain repellency properties or containing a specific PFAS brand or individual compound. To extract the PFAS, a small sub-sample (5 x 5 cm) was further cut into small pieces, placed into a 15 mL plastic centrifuge tube, spiked with mass-labelled internal standards and 10 mL of methanol added. Samples were sonicated for 30 min and centrifuged to remove suspended particulate. Immediately prior to analysis a small aliquot was transferred to the autosampler vial and combined with an equal volume of MilliQ water to ensure good analyte chromatography. Samples were analyzed by LC-MS/MS using the scheduled MRM algorithm. 27 analytes were monitored covering perfluorinated sulfonates and carboxylates, fluorotelomer sulfonates, fluorinated ether acids, and fluorotelomer dialkyl phosphates (diPAPs). Results showed that most of the samples textiles contained at least one PFAS compound, although the number of individual compounds and the PFAS profile varied. PFAS were less frequently detected in the food contact papers, and their levels were comparatively lower.

**4.14.P-Tu119 Ongoing Monitoring Efforts of Per- and Polyfluoroalkyl Substances (PFAS) Across the State of Florida**

***Camden G. Camacho*** *and John A Bowden, University of Florida*

Florida's rich water sources are vital for drinking water; agriculture and other industry; recreation and tourism; ecosystems; and climate resilience. As such, the monitoring of water quality is critical for the state, with consequences for both human health and the economy. Therefore, it is crucial to establish monitoring efforts capable of providing information regarding the health of our water systems to ensure that water is safe for the ecosystem and human health. The present work describes our ongoing efforts toward monitoring per- and polyfluoroalkyl substances (PFAS) within a myriad of water systems in Florida, to establish a PFAS baseline and determine hotspots for establishing priority areas for prospective aquatic ecological assessments and/or remediation efforts. Fresh and saltwater surface samples were obtained from over 2,600 sites via students and citizen scientists from all 67 counties in Florida. Per batch (200 surface water samples per batch), samples were spiked with isotopically labeled internal standards and analyzed using a robust sampling and monitoring workflow (92 PFAS). The samples were subsequently extracted (250 mL) via weak anion exchange solid phase extraction. Each extract, quality control sample (spiked surface water), and extraction/field blank were analyzed using a Thermo Scientific Vanquish ultra-high performance liquid chromatograph coupled to a TSQ Quantis triple quadrupole mass spectrometer, in negative mode using selected reaction monitoring. Derived data was organized by GPS (sub-organized by county). Previous PFAS efforts in Florida have primarily been focused on fresh/drinking water and mostly in areas of known contamination (via aqueous film forming foam release), such as military and aviation facilities. This work expands the current PFAS monitoring efforts to encompass the entire state, including sampling in the marine environment. This expansion presents the potential to identify understudied exposure pathways and areas of concern, leading to an enhanced understanding that can be factored into current coastal regulatory and management efforts.

**4.14.P-Tu120 Seasonal occurrence and distribution of per- and polyfluoroalkyl substances in an urbanized tropical estuarine embayment: San Juan Bay, Puerto Rico**

***David Katz***[1]*, Julia Sullivan*[2]*, Alana Hanson*[1]*, Ivelisse Cappielo-Cosme*[3]*, Evelyn Huertas*[1]*, Thomas Boving*[4]*, Marirosa Molina*[1] *and Autumn Oczkowski*[1]*, (1) U.S. Environmental Protection Agency, (2) Oak Ridge Associated Universities, (3) Oak Ridge Institute for Science and Education (ORISE) participant at U.S. Environmental Protection Agency, (4) University of Rhode Island*

Per- and polyfluorinated alkyl substances (PFAS) are a large class of organic contaminants associated with both point and non-point source contamination. Most environmental reports of PFAS are from temperate locations and estimates of PFAS exposure in the tropical environment are limited, where climatic and socio-economic differences from temperate locations may pose an increased ecological or human health risk. In this study, we quantified a targeted list of legacy PFAS compounds and total PFAS using the total oxidizable precursors (TOP) method during the dry and rainy seasons in surface waters of San Juan Bay, Puerto Rico to better assess the risk these compounds may pose to human and ecological health in a tropical estuary. PFAS were present throughout the estuary, with the lowest values occurring at points with the shortest hydraulic residence time and highest values typically occurring nearest to the tidally restricted lagoons. Targeted ∑PFAS concentrations ranged from 0.9 – 25.9 ng/L during the dry season and 1.9 – 32.9 ng/L during the rainy season. However clear differences in individual PFAS concentrations were observed between seasons; at many stations ∑PFAS concentrations in November 2019 (rainy) were more than double those at the same stations in March 2021 (dry). After the TOP method, higher PFAS concentrations were observed in almost all samples – often a factor of 5 to 10 times higher compared to un-oxidized samples. These large increases post-TOP method underscore the need for comprehensive PFAS analysis, where high concentrations of the dark pool of novel PFAS without authentic standards would otherwise remain unknown. The San Juan Bay estuary system is heavily relied upon by the local population for recreation and livelihood, and chemical contamination potentially exposes an underserved community in the tidally restricted Cano Martin Peña to adverse risk.

**4.14.P-Tu121 Development of Methods and Report on The Detection and Quantification of Chemicals of Emerging Concern from Surface Waters in Southwest Florida**

***Melany Cutie****, Nora Demers and Daniel H Paull, Florida Gulf Coast University*

Endocrine-disrupting compounds (EDCs) are man-made chemicals that can be found in pesticides, plastics, pharmaceutical agents, and personal care products. They travel to nearby bodies of water through wastewater treatment plants (reuse water), septic tanks, fertilizers used for lawn maintenance, and stormwater. Studies have shown that exposure to these chemicals can interfere with growth development, metabolism, reproduction, or the morphology of wildlife and humans. The equipment necessary to analyze these chemicals can be very expensive and difficult to use.

The purpose of my research is to continue to develop and improve more economical methods to detect and quantify what specific chemicals are present in low concentrations in surface waters of Southwest Florida using liquid chromatography – mass spectrometry (LCMS). Liquid chromatography is responsible for separating the components in the water sample, while mass spectrometry offers the identification and quantification of the chemicals present. Water samples collected were prepared for LCMS analysis by first completing solid-phase extraction (SPE) and electrospray ionization (ESI). We will present data on methods using several columns and solvents for the same chemicals and intensive quantitative evaluation. We're developing a methodology that allows the single-quadrupole to provide data that's as specific and as low detection limits as the LC-MS/MS (or triple-quadrupole). We're sacrificing efficiency for specificity/accuracy. For example, it takes 2 hours to do what a triple-quad can do in 10 minutes.

We are collecting water samples from four different types of surface waters locations including 1) community using reuse water for irrigation 2) drainage canals from community using septic tanks for wastewater treatment 3) two lakes on golf courses, one containing reuse water and another that holds stormwater 4) stormwater ponds from the university's campus. Prior research has identified several chemicals including atrazine, bisphenol-S, DEET, sucralose, and triclosan in these waters. We intend to present data from our sampling locations.

Our methods can allow future researchers with limited access to resources and equipment that exists at comprehensive universities such as Florida Gulf Coast University to implement this technique and obtain results more accurately. It also permits us to continue with our research exploring the presence of endocrine disruptors in our local waters.

**4.14.P-Tu122 Per- and Polyfluoroalkyl Substance Mass Release from AFFF-Impacted Soils Under Unsaturated Conditions**

***Stefanie Miller Shea***[1]*, Christopher Higgins*[1] *and Charles E. Schaefer*[2]*, (1) Colorado School of Mines, (2) CDM Smith*

Repeated application of aqueous film-forming foams (AFFFs) to surficial soils have resulted in unsaturated soil systems acting as long term, persistent sources of per- and polyfluoroalkyl substance (PFAS) contamination to groundwater. The processes that control the release of PFASs from the unsaturated zone into groundwater are poorly understood. This study evaluated the mass release of PFASs from two historically AFFF-impacted soils under various saturation conditions. Synthetic rainwater was pumped through 10 soil columns to assess how PFAS mass release was impacted by unsaturated conditions. The column effluents and soils are analyzed for anionic, cationic, and zwitterionic PFASs using high resolution mass spectrometry. Similar to behavior others have observed in saturated soil experiments, chain length and PFAS class impact the rate of release from unsaturated AFFF- impacted soils. Additionally, varying saturation conditions resulted in more PFAS release when compared to constant unsaturated conditions. When the soil was allowed to partially dry before re-wetting, an increase in mass released was observed. This may be due to collapsing air-water interfaces mobilizing more PFASs from the soil.

**4.14.P-Tu123 Evaluating the Chemical Parameters and Potential Impacts to Human Health Risk Assessment from Aqueous Dermal Exposures to Per- and Polyfluoroalkyll Substances (PFAS)**

*Gregory J. Garvey and* ***Graham Ansell****, GSI Environmental, Inc.*

Depending on how PFAS is defined, this group can encompass a very broad range of compounds with a variety of complex functional groups. In addition, a wide range of chemical and physical properties would need to be considered when assessing whether any specific PFAS would cross the transdermal barrier. Moreover, the empirical data for many of these physicochemical properties (*e.g.,* log$K_{ow}$) can be uncertain or limited. Additionally, due to certain properties of PFAS relative to other organics, modeled estimates derived from compilations of values for other organics are unlikely to be predictive for PFAS. As a result, the calculation of human health risks from dermal exposures requires chemical-specific parameters that may be empirically derived or modeled. USEPA RAGS Part E provides screening evaluation methodology that recommends excluding compounds from quantitative evaluation in a human health risk assessment when the dose from dermal contact results in an intake of less than 10% the oral dose. In this evaluation, estimates of exposure and risk from modeled aqueous dermal contact with five PFAS (PFBS, PFHxS, PFOS, PFOA, and PFNA) were evaluated using USEPA methodology and estimates of dermal parameters from the literature. These PFAS are all included in the May 2022 update of the USEPA Regional Screening Levels (RSLs) and include dermal components for tap water exposures. Depending on the log$K_{ow}$ selected for PFOA and PFOS, these compounds may fall outside the Effective Predictive Domain (EPD) recommended by USEPA for quantitatively evaluating dermal exposure. Assigning a unit concentration (e.g., 1 µg/L) for these PFAS in residential tap water results in dermal doses and risks that range from 0.1 to <3% when compared to the ingestion pathway for both child and adult receptors. Moreover, PFAS are not expected to readily cross the transdermal barrier and be dermally absorbed. Consistent with USEPA guidance, for conceptual site models involving both dermal and oral exposure to PFAS, sufficient information is available to exclude the dermal pathway from quantitative risk characterization.

## 4.15 Life at the Fenceline – State-of-the-Science Exposure Assessment for Communities Adjacent to Industrial Facilities

### 4.15.T-01 Introductory Remarks: Exposure Assessment for Communities Adjacent to Industrial Facilities

***Paul C. DeLeo**[1] and William Rish[2], (1) Integral Consulting Inc., (2) ToxStrategies, Inc.*

The Introductory Remarks will set the stage for the session by reviewing the regulatory approach and past precedents regarding the evaluation of human exposures to chemical emissions from industrial and commercial facilities in the United States (U.S.) under the Toxic Substances Control Act (TSCA). Reforms to TSCA were implemented in 2016 with new authorities and mandates for the U.S. Environmental Protection Agency (EPA) to evaluate potential risks of chemicals currently in use in the U.S. Over the past six years with EPA developing its risk evaluation approaches under three different presidential administrations, there have been several significant policy shifts culminating with the January 2022 release by EPA of its "TSCA Screening Level Approach for Assessing Ambient Air and Water Exposures to Fenceline Communities". The Introductory Remarks will review the evolution of EPA's thinking on evaluating exposures to fenceline communities and provide an overview of the January 2022 proposed screening level methodology.

### 4.15.T-02 Review of the Draft TSCA Screening Level Approach for Assessing Ambient Air and Water Exposures to Fenceline Communities Version 1.0

***Daniel Schlenk**, University of California, Riverside*

The United States Environmental Protection Agency (US EPA) held a public virtual meetingof the Science Advisory Committee on Chemicals (SACC) on March 15-17, 2022, to peer review the screening level approach (Version 1.0) for assessing ambient air and water exposures to fenceline communities. The Science Advisory Committee on Chemicals (SACC or Committee), was an outcome of the Toxic Substances Control Act (TSCA) of 1976, as amended by The Frank R. Lautenberg Chemical Safety for the 21st Century Act in 2016. The SACC serves as a primary scientific peer review mechanism of the EPA, Office of Pollution Prevention and Toxics (OPPT). Previous risk evaluations for 10 chemicals conducted under the 2016 amendments to TSCA did not assess exposures to the general population, particularly susceptible subpopulations, including fenceline communities that are near industrial facilities. As an initial step to understanding risks to fenceline communities, EPA developed version 1.0 of a screening methodology and requested input and advice from the SACC on the proposed methodology as well as recommended revisions or improvements to the methodology. A number of charge questions were provided to the SACC and discussed in the March meeting. This presentation will summarize the discussions and the report made public on May 17, 2022. *"The views expressed in the presentation/abstract are those of the author/presenter and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency."*

### 4.15.T-03 Evaluating Exposure and Risk in Fenceline Communities: The Uses and Limitations of Publicly Available Geographic Information System (GIS)-Based Tools

***Ari S. Lewis**, Julie C. Lemay and Naomi L. Slagowski, Gradient*

Federal and state agencies have dedicated significant resources to developing GIS mapping tools that can identify fenceline communities, particularly those that may have environmental justice concerns. Given core environmental justice principles are built on ensuring equitable environmental laws and facilitating public engagement, most of these tools are publicly accessible with user-friendly interfaces that can help the public visualize selected environmental and socioeconomic attributes of a given area. These tools, which include US EPA's EJSCREEN, the US EPA's Risk-Screening Environmental Indicators (RSEI) model, the Toxics Release Inventory (TRI) Toxics Tracker, US EPA's new AirToxScreen Mapping Tool, and the White House Council on Environmental Quality's (CEQ's) new Climate and Economic Justice Screening Tool, have overlapping data sources and cross talk with each other but provide unique perspectives on the intersection of chemical exposures and measures of socioeconomic status (SES) in fenceline communities. This presentation will provide an overview of the various resources that exists for examining exposures for fenceline communities, as well as underscore some of the limitations in using those tools to achieve a refined understanding of potential risk to fenceline communities.

**4.15.T-04 Improving Community Exposure Assessment Using Personal and Local Monitors**
***LeeAnn Racz**, ToxStrategies, Inc.*
The US Environmental Protection Agency has recently issued a draft screening methodology to evaluate potential exposures to fenceline communities from chemicals undergoing risk assessment under the Toxic Substances Control Act. For ambient air exposures, this methodology includes the use of Toxic Release Inventory (TRI) data and AERMOD modeling. Although the methodology does not directly account for cumulative exposures to communities with environmental justice concerns, it allows for the possibility of expanding its use for such purposes in the future. The ability to understand actual exposures to air pollutants is dependent on the ability to measure concentrations at which individuals and communities are exposed. Regional air pollution monitors and models using actual facility emission rates provide better exposure estimates than estimates based on TRI data and a screening model. However, these estimates do not account for factors that can affect true exposures in neighborhoods, such as hot spots, human activity patterns, time indoor versus outdoor, and building terrain effects.

This presentation will discuss how the state-of-science for subpopulation exposure assessment incorporates collecting data with affordable personal and local sensors deployed throughout neighborhoods to capture a finer resolution of cumulative exposure data. It will show how widespread use of such next-generation monitors, coupled with internet-enabled data management, can be combined with data from source emitters, facility fenceline monitoring, and regional monitoring to significantly improve prediction and assessment of community level exposure and cumulative risks.

Examples such as the Breathe London project where mobile personal air quality monitors deployed throughout London identified localized areas of higher concentrations of criteria air pollutants that stationary monitors and models failed to detect, and California's Refinery Fenceline and Community monitoring programs will be discussed. Data from programs like these combined with meteorological data and modeling may be used to guide actions to reduce exposures at the local scale.

**4.15.T-05 Contaminated Sites and Indigenous Peoples in Canada and the USA- A Scoping Review**
***Katie Chong** and Nil Basu, McGill University, Canada*
Indigenous communities in Canada and the USA are disproportionately exposed to contaminated sites, often due to industrial activities adjacent to Indigenous lands. Contaminated sites pose unique challenges to many Indigenous peoples who consider the land as an integral part of food systems, language, culture, and spirituality. Federal management of contaminated sites near 'fence-line' Indigenous communities is challenged by epistemological differences, regulatory barriers, and a lack of scientific research to support community-specific exposure assessment. While pollution in general has been well-studied in the context of Indigenous peoples, there is limited information available on the unique issue of federal contaminated sites, and a lack of coordination between industries, governments, scientists, and Indigenous communities. Most studies are focused on a local scale, which are important, but evidence synthesis is also needed to inform decision-making. Thus, we conducted a scoping review to identify and map available information on contaminated sites and Indigenous peoples in Canada and the USA, utilizing three streams of data retrieved from January-March 2022: a systematic literature search (key word groups: *Indigenous people* and *contaminated sites*); a grey literature search; and an analysis of federal contaminated site data (Canada's Federal Contaminated Sites Inventory (FCSI) and US EPA's Superfund Database). Our search yielded 49 peer-reviewed articles, 17 grey literatures, and 8114 federal site records (1236 Superfund, 6878 FCSI), allowing us to summarize the state-of-the-science on the exposure of 'fence-line' Indigenous communities to contaminated sites, management strategies, and the

inclusion of Indigenous peoples in site processes. The results spanned many disciplines, revealing the contamination of the lands of 815 distinct Indigenous tribes and nations and the presence of 440 different contaminants or contaminant groups found at 4976 contaminated sites. By integrating three diverse data streams we discovered a disparate body of information, pointing to the need to prioritize holism, efficiency, and Indigenous leadership in site assessment, management, and research. This should include re-thinking community-specific risk assessments to better understand Indigenous conceptualizations of human and ecological health, and greater collaboration between the scientific community, Indigenous leadership, and federal governments.

**4.15.T-06 Target Organ Toxicity Using Histopathological and Biochemical Changes in a Rat Model Following Oral Exposure to Contaminated Groundwater from an Industrial Site.**

***Bright Boamah**[1], Steven Siciliano[1], Natacha S. Hogan[1], Markus Hecker[1], Mark L. Hanson[2], Ahmad Al-Dissi[1], Rachel Peters[3], Aditya Manek[1] and Lynn Weber[1], (1) University of Saskatchewan, Canada, (2) University of Manitoba, Canada, (3) Federated Co-operatives Ltd., Canada*

Groundwater containing a complex mixture of known and unknown contaminants was collected from an industrial site for the characterization of toxic effects. The first study, a time-course study involved an oral exposure to a single concentration (0.05% v/v) of groundwater compared to control (7, 14, 28, and 56 days exposure) in male Sprague Dawley rats (n=10/group). The second study involved a 60-day oral exposure to drinking water (control group), 10% v/v low impact water from an alternate well on-site compared to 0.01% v/v, 0.1% v/v, 1% v/v and 10% v/v of high impact groundwater concentration (n= 5 males and 5 females/group). The dose-response study showed evidence of reduced alpha-2 macroglobulin between the negative control group and the low and high impact groups. The dose-response experiment showed dose-independent acute tubular necrosis in rats exposed to water from the alternate well and the high-impact well. However, plasma symmetric dimethylarginine was significantly elevated in the dose-response study following exposure to 1% and 10% v/v high impact groundwater as well as the time-course study on day 14. The dose-response further showed cerebral blood flow directly correlated to the exposure concentrations of high-impact groundwater. Conversely, diminishing rotarod performance test was associated with increased high impact groundwater concentration. The time-course study showed gonadotoxicity in the form of reduced epithelial height in the seminiferous tubules in the exposed groups within all the time points. Additionally, the cellular progression and development of spermatogonia and supporting cells were significantly reduced within all the exposed groups as quantified by the Johnsen scores. In summary, time-course and dose-response studies showed evidence of immunotoxicity, and nephrotoxicity. The time-course study showed gonadotoxicity, while the dose-response which used higher doses showed neurotoxicity. Based on the response pattern, hydrocarbons are suspected to be the class of contaminants driving toxicity in the groundwater samples and will be confirmed with further analyses.

**4.15.T-07 Estimating the Health Risk Associated with Metal Exposure at Agbogbloshie E-Waste Recycling Site and the Surrounding Neighbourhood in Accra, Ghana**

***Matt Dodd**[1], Lydia Amponsah[2], Steve Grundy[1] and Godfred Darko[2], (1) Royal Roads University, Canada, (2) Kwame Nkrumah University of Science and Technology, Ghana*

Electronic and electrical waste (e-waste) has been described as the fastest growing domestic waste stream in the world. According to the Global E-Waste Monitor, of the 53.6 million metric tonnes of e-waste generated in 2019, only 17.4% was collected and recycled. A portion of the unaccounted-for waste ends up in the informal recycling stream. Until recently Agbogbloshie in Accra, Ghana was used as an informal e-waste recycling site including dismantling, and open burning of e-waste and other wastes. Soils at Agbogbloshie have been shown to contain toxic metals at levels that can impact the health of recyclers and occupants. This study was conducted to assess the distribution and potential health risk associated with toxic metals in surface soils in the surrounding community. Surface soil samples were collected at the dismantling sites (n=11), burning areas (n=14) and along a 300 m x 300 m grid in the surrounding community (n=64). Total metal concentrations were determined by XRF and ICPMS and an in vitro bioaccessibility assay was used to estimate gastric metal bioavailability. The data obtained confirmed the presence of elevated concentrations of potentially toxic metals at the dismantling and burning sites. Mean concentrations at the burning sites were As:218; Cd: 65; Cr: 182; Cu: 15,841; Ni: 145; Pb: 6,106; Sb: 552; and Zn: 16,065 mg/kg while the dismantling sites had mean concentrations of As: 23; Cd: 38; Cr: 342; Cu: 3239; Ni: 96; Pb: 681; Sb: 104; and Zn: 1658 mg/kg. Geoaccumulation indices and enrichment factors corroborated the presence of elevated levels of metals at the recycling sites. The spatial distributions and concentrations of elements including As, Cd, Cu, Ni, Pb, Sb and Zn in the surrounding community showed these elements were elevated at various locations and exceeded international environmental soil quality guidelines. The mean bioaccessibility values included As (23%), Cu (59%), Pb (68%), Ni (38%), Sb (14%) and Zn (58%). The inclusion of bioaccessibility data in the risk estimation resulted in decreases in the individual metal hazards, albeit the risk associated with the ingestion of soil-borne metal contaminants at the e-waste dismantling and the burning areas was very high. Although there was evidence of increases in metal concentrations in the areas closer to the burning and dismantling sites, the human health risk associated with soil ingestion was considerably lower in the surrounding neighbourhood.

**4.15.T-08 Risk Assessment of Berry Health in Alberta Oil Sands Region.**

***Chanel Yeung** and Mandy Olsgard, Integrated Toxicology Solutions Ltd., Canada*

The oilsands berry monitoring program is a multi-community Indigenous knowledge-based monitoring (CBM) program in the Athabasca Oil Sands Region (AOSR) led by five Indigenous communities: the Fort McKay First Nation, Fort McKay Métis Nation, Fort McMurray #468 First Nation, Fort McMurray Métis Local #1935, and the Conklin Métis Local #193. The project was initiated by Fort McKay communities in 2011 based on concerns of the declining health and abundance of berries at local patches surrounded by operating oil sands mines (8) and in situ facilities (10). The program objective is to answer community questions related to the consumption risks and observed changes in the abundance and quality of berries in culturally significant harvesting areas which may be impacted by chemical emissions from oil sands development. Western science components (including social science) involve development of standard sampling procedures, analyzing measured concentrations of metals, polycyclic aromatic hydrocarbons and health promoting compounds to understand spatial and temporal differences and trends, comparisons between commercial and local berries, and the effects of washing. Western science results are presented to community members for feedback and verification as well as incorporation of IK recommendations to modify and adapt the berry CBM and address new questions which may have arisen based on monitoring and risk assessment results. The CBM is providing useful data to both traditional land users in understanding potential health risks from consuming local berries and western scientists in understanding impacts from different types of oilsands developments (surface mines vs. *In situ*). The presentation will describe how the program has evolved over the past decade, development and results from the Indigenous community specific human health risk assessment, and present key results related to differences between harvesting locations and potential risks from consumption of locally harvested berries in each communities' traditional territory.

**4.15.P Life at the Fenceline – State-of-the-Science Exposure Assessment for Communities Adjacent to Industrial Facilities**

**4.15.P-Mo126 An advanced fugacity model by incorporating computational fluid dynamics to predict indoor behavior of an insecticide for aerosol spray**

***Sayuri Tanaka**[1], Hiroaki Kondo[2], Yoshihide Matoba[1] and Tomohiko Ihara[3], (1) Sumitomo Chemical Co., Ltd., Japan, (2) National Institute of Advanced Industrial Science and Technology, Japan, (3) The University of Tokyo, Japan*

We are now advancing the fugacity model (Mackay et al. 1982) by incorporating computational fluid dynamics (Patanker S. V. 1980). The fugacity model is able to effectively estimate the transports of a chemical substance between several environmental compartments and thus, it is widely used to describe environmental behaviors of chemical substances (Tao, S. et al. 2003). However, the model assumes concentration of a substance in each compartment is uniform and hence, it cannot predict distribution of the substance in each compartment. Our purpose is to develop a new model for predicting not only the transports of a substance between environmental compartments simulated using the fugacity, but also distributions of the substance in each compartment simulated using numerical models. In this study, we tried to develop a new model to predict time-dependent behaviors of an insecticide sprayed by an aerosol canister in a room. Matoba et al. (1993) had developed a fugacity model to describe settlement of aerosol droplets to the floor as well as time-dependent concentrations of an insecticide in the droplets and air, on the floor, wall and ceiling. However, they did not describe precise movements of the droplets affected by air flow and the gravity, or time-dependent distributions of the insecticide in the air and on the floor, wall and ceiling. We used computational fluid dynamics to describe movements of the droplets from the air to the floor, wall and ceiling as well as the airflow in the room, and used the fugacity to simulate interactions among the droplets, air, floor, wall and ceiling. We will show comparisons in concentrations of the insecticide between the simulations and actual measurements at the SETAC meeting. The advanced numerical model would be helpful to understand precise behaviors of the insecticide under various environmental and usage conditions without performing actual experiments.

**4.15.P-Mo127 Tiered Screening Level Assessment Protocol and Sensitivity Analysis for Rapid Risk Prioritization of Potential Emissions to Ambient Air in Fenceline Communities**

***Rachel Young*[1]*, Kenneth Unice*[2]*, Stephanie A. Thornton*[2]*, Laura H. Allen*[2]*, Paige M. Wells*[2] *and Marisa L. Kreider*[2]*, (1) Stantec Consulting Services Inc. , (2) Cardno ChemRisk now Stantec*

The U.S. EPA recently proposed a draft Toxic Substances Control Act (TSCA) screening approach for stack and fugitive emissions to the ambient air of fenceline communities. The proposed methods were evaluated for consistency with prior agency risk assessment guidance. The proposed approach, which focused on generic source parameters and Toxic Release Inventory (TRI) submissions, was not coordinated with other agency programs addressing ambient air quality such as the National Air Toxics Assessment, and lacked consideration of reasonably available site-specific information, a tiered approach for refinement, as well as a transparent model evaluation, including a sensitivity analysis. These inconsistencies between the proposed and existing risk assessment approaches suggest a need for rapid assessment by affected facilities to help inform transparent risk communication and sound risk management engagement with Agency and community stakeholders including consideration of cumulative or aggregate exposures. This work presents a stepwise, rapid assessment, three-tier modeling approach consistent with Agency guidance for the efficient development of risk prioritization and stakeholder engagement programs. The approach is illustrated through the prediction of ambient air concentrations associated with representative small, medium, and large tonnage facility source configurations reported in the National Emission Inventory. Tier 1 consists of a precautionary AERSCEEN assessment with few site-specific information requirements intended to understand the need for more time intensive site-specific modeling. Tier 2 includes the implementation of a refined AERMOD assessment including site-specific source parameters, terrain, and meteorology. Additional refinements such as the consideration of seasonal facility operational conditions, human activity patterns (e.g., fraction of day, month, year or lifetime exposed), or monitoring data are considered when necessary in a final Tier 3 refinement step. The decision criteria for advancing between each of the three tiers and contextualization of key determinants in the hierarchy are evaluated using both qualitative and quantitative sensitivity analyses. Finally, differences between the U.S. EPA proposed approach and this three-tier approach are interpreted. Findings developed in the three-tier approach are expected to be useful for risk communication and management.

**4.15.P-Mo129 Assessment of Particle Counts in both Indoor and Outdoor Environments in Allegheny County, PA**

***Abdul Alobireed*[1]*, Samantha Totoni*[1,2]*, Linda Wigington*[2]*, Donald Fugler*[2] *and Nesta Bortey-Sam*[1]*, (1) University of Pittsburgh, (2) Reducing Outdoor Contaminants in Indoor Spaces (ROCIS)*

**Introduction:** Air pollution (contamination of indoor and outdoor environment) is one of the leading causes of mortality and the greatest environmental risk to health, with a disproportionate impact on vulnerable populations. Globally, low- and middle-income communities, part of many Environmental Justice (EJ) issues, are exposed to a higher burden of air pollution. Moreover, industrial facilities are more likely to be found in or closer to some of these communities. Allegheny County ranks 16th on the list of most polluted counties by year-round particle pollution and Pittsburgh ranks 8th on the list of U.S. cities most polluted by year-round particle pollution 2016—2018.

**Objectives:** The objective of this study is to assess the levels, distribution, and seasonal variations of particle counts in indoor and outdoor environment in Allegheny County with respect to air pollution.

**Methods:** Between January 2016 and August 2021, real-time Dylos particle counts (>0.5 microns) data were collected, through the Reducing Outdoor Contaminants in Indoor Spaces (ROCIS) initiative, from indoor and outdoor spaces from over 290 homes in 14 Allegheny County municipalities. Particle counts were continuously monitored as 1-minute interval for a period of three weeks in each indoor and outdoor spaces, simultaneously, throughout the year. QGIS software was used to geocode the participants proximity to some potential source(s) of pollution and EJ Census Tracts.

**Results:** Based on particle counts, the indoor data from three municipalities (representing 21%) and the outdoor counts from all fourteen municipalities (100%) had relatively poor air quality (total particle count range of greater than 1050, or roughly equivalent to PM2.5 of 8 ug/m$^3$). The data showed seasonal variations with significantly higher outdoor particle counts during winter followed by fall compared to spring and summer. Moreover, the mean indoor counts exceeded the mean outdoor counts in only two municipalities. Behavioral changes, such as cooking methods and/or smoking, play a role in the levels of indoor particle counts. Industrial activities, sometimes located around low income and minority communities, could be another major contributor to the outdoor particle counts measured.

**Conclusion:** Despite progress in reducing air pollution at both national and local levels since 1970s, particle count in both indoor and outdoor environments show poor air quality in some homes in Allegheny County.

**4.15.P-Mo130 The PFAS Chicken and Egg Dilemma: A Probabilistic Analysis of PFAS Exposure and Risk via Consumption of Home-Produced Poultry and Eggs**

***James K. Wilhelm***, *Gregory J. Garvey, Philip E. Goodrum and Janet K. Anderson, GSI Environmental, Inc.*

Due to their grazing habits and increase in popularity, home-produced (domestic) chickens represent a potential dietary exposure pathway for environmental contaminants from consumption of eggs and edible tissues (e.g., breast, wings, thighs). Of growing concern in some residential communities is the extent to which exposure to per- and polyfluoroalkyl substances (PFAS) may occur through the consumption of home-produced foods. Some PFAS warrant quantitative evaluation in risk assessment due to their persistence in soil and water used for irrigation, bioaccumulation potential, and toxicological profiles. In recent years, researchers have examined differential uptake of certain PFAS into livestock that may be exposed directly via consumption of PFAS in soil and water, as well as indirectly via trophic transfer from dietary items such as plants and invertebrates. This study quantifies the average daily dose (ADD) and human health risk for these pathways when domestic chickens are raised in soil contaminated with PFOA and PFOS. Using Monte Carlo Analysis, two food web models are applied in a probabilistic risk assessment. The first model quantifies the variability in concentrations in eggs and muscle given literature-based estimates of dietary preferences, food ingestion rates, and body weights of domestic chickens, coupled with experimentally-derived intake slope factors to estimate tissue concentrations of PFOA and PFOS. The second model incorporates national survey data on age-specific, consumer-only consumption rates among U.S. populations to derive a probability distribution of average daily doses. Current USEPA chronic oral reference doses (RfDs) for PFOA and PFOS (based on subchronic RfD's from ATSDR) were applied to evaluate risk ranges expressed as distributions of hazard quotients (HQ) for young child residents. Results indicate that background levels of PFOA and PFOS in residential soil in the U.S. are well below concentrations that would exceed risk management HQ thresholds for children, and that variability in total ADD for both compounds is dominated by egg consumption rates. For sites with elevated PFOA and PFOS in soil and water, this probabilistic modeling approach may be used in a comprehensive risk characterization, as well as to establish risk-based soil action levels or consumption advisories protective of communities consuming home-produced poultry and eggs.

**4.15.P-Mo131 PFAS in Home-Grown Produce: Methods for Food Intake Modelling and Risk Assessment**

***Felipe Gustavo Becker****, Gregory J. Garvey, James K. Wilhelm and Philip E. Goodrum, GSI Environmental, Inc.*

The increase in home gardening activities across the country during the SARS-CoV-2 pandemic has generated renewed interest in determining if homegrown foods could pose potential risks to consumers in areas of known soil and water contamination. Food consumption preferences across the U.S. vary widely from region to region, and recent data on food intake rates between urban, suburban, and rural populations are limited, making exposure estimations difficult for unique communities. Over the past decade researchers have examined differential uptake of a wide range of per- and polyfluoroalkyl substances (PFAS) into edible portions of plants such as fruits and vegetables in order to gauge their bioaccumulative potential and estimate likely exposure concentrations. In this study, we demonstrate how this rich database can be combined with national and regional information on food ingestion rates in order to rank order commodities that may contribute most to total PFAS exposure. Data on home-grown food consumption was obtained from three sources – the U.S. Exposure Factors Handbook, U.S. EPA's online ExpoBox tool, and reports summarizing state-wide and regional growing practices. Information from local and regional farming reports includes listings of fruits and vegetables commonly grown in backyard gardens in the Northeast. These data, along with literature on soil-to-plant bioaccumulation factors (BAFs) can be paired using the categories corresponding to the Food Commodity Intake Database (FCID). The product of the food ingestion rates and matched with bioaccumulation factors (BAFs) serves as a weighting factor to rank order the key home-produced foods that are potential sources of exposure to PFOA, PFOS, and other PFAS in the U.S. Information is presented both as point estimates and probability distributions, which would support evaluations of human health exposure and risk in a probabilistic risk assessment framework.

**4.16.P Non-Targeted Analysis of Environmental Contaminants: Methods and Applications**

**4.16.P-Tu124 Detecting and Identifying Source-specific Water Contaminants in the South Florida's Miami River**

***Kassidy Troxell*** *and Piero Gardinali, Florida International University*

The historic Miami River in South Florida is sourced from Everglades-based freshwater that has been drastically changed over time after a rapid development and industrialization of its surrounding areas raising concerns about the possible contamination of the urban water system (UWS) and the quality of water discharged into its endmember Biscayne Bay. Biscayne Bay requires freshwater inputs to maintain its ecological balance but has been disrupted by canals and other water management operations that have negatively impacted the ecosystem, resulting in two fish kills in 2020 and 2021 totaling over 27,000 fish deaths. Therewith, it is important to assess how the freshwater from the Everglades changes throughout the UWS in the Miami River to fingerprint the pollution sources affecting the water discharged into the bay. Natural and anthropogenic sources contribute a unique signature of elements and chemicals into water, creating a "water fingerprint". This project has generated a database of physical and chemical water properties for the Miami River by developing and applying methods for detection and quantification of natural and anthropogenic contaminants in freshwater (Everglades), UWS (Miami River), and coastal (Biscayne Bay) endmembers. The assessments included traditional water parameters (conductivity, pH, DO, turbidity), water optical properties (FDOM, CDOM, chlorophyll, 3D EEMs), elemental and molecular composition (nutrients, hydrogeochemical tracers, and metals), and the analysis of target and non-target (NTA) organic contaminants using high-resolution mass spectrometry. Natural and anthropogenic components were traced as they move through this interconnected system and were observed to be affected by specific localized emissions. Sucralose, an artificial sweetener and known wastewater tracer, has been traced from the Everglades increasing in concentration throughout the Miami River (>500ng/L) and declining as the water discharges to Biscayne Bay (<200ng/L), attributed to dilution. Similar trends were observed with endocrine-disrupting compounds (carbamazepine and caffeine), metals (Cr, Mn, Fe, Zn, and Al), and nutrients (TP, SRP, TN, NH3/NH4-N, NO2-N, and NO3-N). NTA chemical space fingerprints and tentatively identified compounds support the presence of anthropogenic sources in this UWS. Understanding the pollution sources' impacts on rivers and canal systems are crucial to understand and guide efforts to save and restore Biscayne Bay.

**4.16.P-Tu125 Changes in metabolome and neurotransmitter profile of larval zebrafish after developmental exposure to Aroclor 1254**

***Corey Green**, Ana Alonso, Jean-Christophe Cocuron and Aaron Roberts, University of North Texas*

Polychlorinated biphenyls (PCBs) are long-lived synthetic compounds that were widely used until 1979. The neurotoxicity of non-dioxin-like PCBs (NDL-PCBs) has been well documented within mammalian species, however, speculation remains as to the specific underlying mechanisms. Previous research has shown developmental exposure to Aroclor 1254 causes dose dependent tremors in the eyes of larval zebrafish, indicating novel neurotoxic effects. In addition, the neurotoxicity of NDL-PCBs has not been extensively reported in fish. Metabolomics is a method of characterizing cellular activity or stressor response by quantifying concentrations of small molecules, or metabolites. In recent years, metabolomics has expanded beyond simple biomarker quantification toward elucidation of molecular mechanisms. Here, we use targeted and untargeted metabolomics techniques to investigate the metabolomic and neurological effects of exposure to Aroclor 1254 in developing zebrafish. Embryos were exposed at 6 hpf via aqueous solution for 96 hr without renewal. Samples were flash frozen in liquid nitrogen and lyophilized before being processed. Untargeted metabolomic analysis was done using GC-MS techniques and revealed 153 features of which 19 were significantly different between treatments, 5 of which were identified as potential hexoses, amino acids and organic acid. Partial least squares-discriminant analysis (PLS-DA) on untargeted data showed a significant separation between treatments using 95% confidence intervals. To confirm the preliminary differences observed by GC-MS profiling, a LC-MS/MS-based targeted metabolomics analysis was performed to quantify 109 metabolites including amino acids, sugars, organic acids, and phosphorylated metabolites of which 7 had statistically significant differences between treatments. There was clear separation between treatments after PLS-DA analysis of targeted data. Aroclor 1254 treatment had significantly higher organic acid and phosphorylated compounds. Finally, neurotransmitters including precursor and derivatives, were quantified using LC-MS/MS. These findings are important in relating known neurotoxic endpoints to underlying mechanisms of PCB toxicity in early lifestage fishes.

**4.16.P-Tu126 Compositional Characterization and Fingerprinting of a Diverse Library of 218 Crude Oil Samples Using Ion Mobility Spectrometry-Mass Spectrometry**

***Alexandra Christian Cordova**[1], Alina Roman-Hubers[1], Fred Wright[2], Thomas McDonald[1], Weihsueh Chiu[1] and Ivan Rusyn[1], (1) Texas A&M University, (2) North Carolina State University*

Exposure assessment, a critical step in risk assessment, is a challenge with substances as complex as crude oils and other substances of unknown, variable, complex, or biological (UVCB) composition. This is especially true in time-sensitive circumstances such as spills and disasters, where spilled products are subject to rapid environmental alteration. Conventional analytical techniques such as gas chromatography-mass spectrometry (GC-MS) have considerably advanced our understanding of oil chemistry to enable chemical "fingerprinting" and origin tracing; however, these are time-intensive methods typically used for whole-substance evaluation or targeted analysis of predefined constituents of concern. Recent studies have found that analytical techniques with improved resolving power and run times such as ion mobility spectrometry-mass spectrometry (IMS-MS) facilitate fingerprinting and untargeted characterization beyond common petroleomic practices. However, prior studies lack diversity of sample location and in-depth chemical characterization. Thus, the current study utilizes targeted gas chromatography-mass spectrometry (GC-MS) and untargeted ion mobility spectrometry-mass spectrometry (IMS-MS) data to fingerprint and characterize 218 crude oils. The oils were separated into 11 categories based on origin and availability of samples: 1 category of non-US samples, 2 categories for domestic onshore samples, and 8 categories differentiating offshore oils in the Gulf of Mexico by leasing area. We hypothesized that oils belonging to the same category (i.e. originating from similar locations) would have the most similar fingerprints and chemical compositions. Oils were characterized by coupling IMS-MS data acquisition with Kendrick Mass Defect analysis, a novel workflow enabling molecular formula assignment to most abundant constituents. Chemical signatures from each group were then evaluated by unsupervised hierarchical clustering and the predictive efficiency of the categories was determined by adapting supervised prediction analysis of microarrays (PAM). Overall, the results of this study are three-fold: (1) IMS-MS data facilitated fingerprinting and characterization to a greater extent than conventional GC-MS data; (2) the assigned categories exhibited high accuracy of prediction, even with a large sample size; and (3) the "biomarker" molecules driving categorical signatures were identified to enable their use as a rapid screening tool for unknown samples.

**4.16.P-Tu127 Non-Targeted Analysis of Environmental Contaminants in Tijuana River Estuary via Composite Integrative Passive Sampler**

***Kesten Bozinovic***[1]*, MacKenzie King*[2]*, Damian Shea*[3] *and Goran Bozinovic*[4]*, (1) Boz Life Science Research and Teaching Institute, (2) Georgetown University, (3) Statera Environmental, Inc., (4) University of California, San Diego*

Cross-border flow of untreated sewage and other sources of pollution from Mexico to the US through the Tijuana River Estuary (TRE) watershed is an international human and ecological health hazard. Prioritization and mitigation of these hazards is hampered by a lack of knowledge of the identity and quantity of chemicals already present in the TRE and what is currently being discharged across the border.

We investigated the presence and concentrations of chemical pollutants in the TRE by an exploratory qualitative non-target analysis (NTA) for over 900,000 chemicals in the US EPA CompTox Chemicals Dashboard database using high resolution GCMS and LCMS, and by quantitative targeted analysis of over 150 organic chemicals using GCMS and LCMSMS. This presentation focuses on the NTA analytical workflow applied to sediment samples and to a novel passive sampling device deployed in water, the Composite Integrative Passive Sampler (CIPS), that quantitively accumulates both polar and non-polar organic chemicals and provides a time-weighted-average concentration over the time of deployment.

The CIPS were deployed for 2-3 weeks, and sediment samples were taken at four sites in the San Diego portion of the TRE during two dry and wet seasonal events. Chemicals identified with $\geq 85\%$ match factor in the NTA were considered for analysis. The site at the US-Mexico border had the highest number of identified chemicals and the highest relative abundance of MS response for both CIPS and sediment. Most of the chemicals detected across all CIPS sites are commonly used in industrial processes. Many PAHs and PAH degradation products were identified along with current and legacy chlorinated pesticides and consumer product chemicals (e.g., caffeine and ibuprofen). There were no obvious differences between wet/dry events, though previous reports suggested higher pollution resulting from overflow during wet events. The border site had the highest occurrence of PAHs, phthalates, and current use and legacy chlorinated pesticides.

This study is the first exploratory NTA of what organic chemicals are present in the water and sediment and is unique in applying the novel CIPS passive sampling device to NTA methodology. This work helps to establish priorities for targeted quantitative analysis and forms the basis for future pollution assessments necessary to effectively identify and mitigate infrastructure difficulties.

**4.16.P-Tu128 Identifying Children's Exposure to Chemicals Through Soil and Dust Ingestion Using Non-Targeted Analysis Approaches**

***Joseph Cox**, Danni Cui, Daniel Bagner, Piero Gardinali, Natalia Soares Quinete and Emily Mejias, Florida International University*

Children are vulnerable to environmental exposure of contaminants due to their small size, lack of judgment skills, as well as their proximity to dust, soil, and other environmental sources. Previous studies have often measured silicon, aluminum, and titanium in children's feces as tracer elements to estimate the rate of soil and dust ingestion, making assumptions that these elements are not absorbed by the gastrointestinal tract and do not account for dietary intake through other non-food items, such as toothpaste and medicine. A better understanding of the types of contaminants that children are exposed to or how their bodies remove these compounds is needed. This research study aims to implement an innovative methodology based on non-targeted analysis (NTA) screening approaches to identify specific chemical tracers of dust and soil exposure. To evaluate child exposure, families with children between 6 months and 5 years of age were recruited to provide urine, dust, soil, food, and water samples for non-targeted analysis. We have developed different sample preparation methods which included online solid phase extraction, accelerated solvent extraction, and QuEChERS followed by liquid chromatography-high resolution mass spectrometry (LC-HRMS) using a Q-Exactive Orbitrap MS system in electrospray ionization (ESI) mode. Samples were run in full scan negative and positive mode with a scan range from 100.0 to 800.0 m/z at a resolution of 140,000, followed by data dependent MS/MS with a normalized collision energy of 30 and at a resolution of 35,000. Post-processing of the raw data files was performed using the software Compound Discoverer (CD) 3.3. After peak picking, blank subtraction, molecular formula generation, isotopic pattern comparison, adducts evaluation, assignment and comparison of fragment pattern, and database searching, a list of features was generated with chemical name, molecular weight, and retention time. Identified features were plotted using Kendrick mass defect plot and Van Krevelen diagrams to show unique patterns in different samples and space regions of anthropogenic compound classifications. The preliminary results of this study will present new NTA methodologies for assessment of a variety of sample types, provide detailed information on environmental chemicals of potential health concern in these samples, and potentially identify specific candidate tracers for soil and dust ingestion by young children.

**4.16.P-Tu130 Evaluation of Linear Response in Non-Target MS/MS Data is a Useful Quality Control Metric for Human Exposure-Relevant Matrices**

***Kirsten Elise Overdahl** and Alan K. Jarmusch, National Institute of Environmental Health Sciences (NIEHS)*

Post-processing cleaning of non-target mass spectrometry data is necessary. However, whereas targeted analysis utilizes well-established quality control (QC) processes to validate variability and linearity of detected chemicals, these metrics do not easily translate to non-target methods. To address the challenge of quality control in non-target mass spectrometry data, we implemented system suitability metrics across a breadth of non-target projects. In this presentation, we characterize and evaluate an assessment of feature linearity in NIST Standard Reference Materials (SRM) 1950, 2585, 3672, 3673, 8231, and corresponding experimental samples (serum, plasma, urine, and house dust). We used reverse phase ultra-high performance liquid chromatography (UHPLC; Thermo Fisher Vanquish Horizon) coupled to high-resolution mass spectrometry (HRMS; Thermo Fisher Orbitrap Tribrid Fusion; electrospray ionization (+/-); AcquireX iterative MS/MS analysis). Compound Discoverer software (Thermo Fisher) was used for initial processing of data and feature annotation; features were then passed through in-house R code via Jupyter notebook for further processing and statistical analysis. Data were collected both from samples and from a pooled quality control (QC). To characterize the linearity evaluation, for each SRM we performed a multipoint calibration evaluation over a larger range of injection volumes and by injecting different concentrations. To evaluate the effect of linearity evaluation on experimental sample analysis, linearity evaluation was performed by multiple injections of the pooled QC at three volumes to acquire a three-point calibration for all features detected. We performed statistical analyses on all linearity evaluations; features shown to be not linear, statistically, over the evaluation range were removed from the dataset. Annotations, chromatography, and origins of features were then evaluated to understand sources of non-linear features. We determined that features removed were contaminants, artifacts of algorithm feature-finding (eg. peak-splitting), and true biological signals but for which the dynamic range of response was non-linear. In all matrices studied, linearity evaluation allowed high-quality annotations to remain. Our results demonstrate that filtering of non-linear features from non-target data improves the quality and understanding of non-target analyses for multiple matrices relevant to human exposure analysis.

## 4.16.V Non-Targeted Analysis of Environmental Contaminants: Methods and Applications

### 4.16.V-02 Non-target and Suspect Screening of Bioaccumulative Organohalogen Compounds in Marine Fish From Seto Inland Sea, Japan

***Akitoshi Goto****, Nguyen Minh Tue, Shinsuke Tanabe and Tatsuya Kunisue, Ehime University, Japan*

Two-dimensional gas chromatograph–time-of-flight mass spectrometer (GC×GC–ToFMS) has been drawing attention as a powerful screening tool for persistent organic pollutants (POPs) and their related compounds. Recent screening studies using GC×GC–ToFMS have reported the accumulation of unmonitored organohalogen compounds (OHCs) in the tissues of apex predators of the marine food web such as marine mammals, but information on the accumulation of such OHCs in lower-trophic level organisms (*e.g*., marine fish and shellfish) is still lacking. Based on this background, our previous study performed non-target screening of POP-like compounds in Japanese bivalves using GC×GC–ToFMS and detected various OHCs including unidentified compounds. The present study aimed to screen known and unknown OHCs in black sea bream (*Acanthopagrus schlegelii*) which preys marine bivalves, to examine their exposure profiles and bioaccumulative potential. Black sea breams ($n$ = 10) were sampled during 2018–2019 from the western part of Seto Inland Sea, Japan. The muscle and liver tissues of a representative specimen, which has the largest body length in the sample set, was used for non-target/suspect screening analyses of OHCs. Briefly, freeze-dried tissues were extracted with acetone/*n*-hexane (1:1 *v*/*v*), and then obtained crude extracts were subjected to gel permeation chromatography (GPC). The GPC-treated solution was analyzed by using GC×GC–ToFMS with electron ionization (EI) mode. OHCs on the two-dimensional total ion current chromatograms (2D TICCs) were identified based on their retention times and mass spectra, which were verified by comparing with those of authentic standards and/or literature data. The results of GC×GC–ToFMS analyses revealed the accumulation of four categories of OHCs in the muscle and liver samples: POPs and their related compounds, chlorinated organophosphate esters (OPEs), halogenated natural products (HNPs), and unidentified halogenated compounds (UHCs). The accumulation profiles of these OHCs were similar in both tissues, and no organ/tissue specific accumulation was observed. However, it was noteworthy that UHCs were detected in not only the representative but also all the other specimens, suggesting chronic exposure against black sea breams inhabiting Seto Inland Sea or bioaccumulative potential.

### 4.16.V-03 Probing Different Wastewater Treatment Processes for Removal of Emerging Contaminants Using Non-Targeted Analysis

***Monika Dubey****, Bhanu Prakash Vellanki and Absar Ahmad Kazmi, Indian Institute of Technology Roorkee*

Wastewater effluent is a significant contributor to emerging contaminants (ECs) in the environment and may cause acute or chronic toxicity, especially for aquatic species. The wastewater treatment systems are designed considering the regulated water quality parameters while ignoring the performance for removing ECs. Non-targeted analysis (NTA) through chromatographic separation coupled with high-resolution mass spectrometry produced datasets can be used to obtain insights into the fate, including transformation and transport of ECs in the wastewater treatment systems. The talk explores the use of NTA to compare the performance of the treatment technologies. In this study, we evaluated the treatment performance of 10 full-scale municipal wastewater treatment plants based on 7 different wastewater treatment technologies in the winter and summer seasons over 2 years. The fate of ECs was ascertained by detecting degradation/transformation by-products using in-built and universal libraries, including NIST, Pubchem, and Chemspider. The result shows that treatment systems with higher hydraulic retention time improved contaminant removal. A pilot-scale biological nutrient removal based wastewater treatment system was also studied to assess the removal and transformation of ECs in anaerobic, anoxic, and aerobic zones. In general, aerobic conditions were found favourable for the removal of contaminants. Further, 16s rRNA amplicon sequencing was performed to study the microbial communities at phylum, class, and order levels under different redox conditions to find a relation between the presence of microbial community with the removal of ECs. The findings from this study can be used to develop contaminant monitoring strategies and optimal biological treatment conditions for removing ECs.

**4.17.P Passive Sampling Methods for Assessing Bioavailability and Toxicity of Insoluble Substances**

**4.17.P-Mo134 Comparing Equilibrium Concentrations of Polychlorinated Biphenyls based on Passive Sampling and Bioaccumulation in Water Column Deployments**

***Robert M. Burgess***[1]*, Mark Cantwell*[1]*, Zhao Dong*[2]*, James S. Grundy*[3] *and Abigail Joyce*[4]*, (1) U.S. Environmental Protection Agency, (2) Harvard T.H. Chan School of Public Health, (3) Oak Ridge Institute for Science and Education (ORISE) participant at U.S. Environmental Protection Agency, (4) Duke University*

Biomonitoring at contaminated sites undergoing clean-up, including Superfund sites, often use bioaccumulation of anthropogenic contaminants by field-deployed organisms as a metric of remedial effectiveness. Such bioaccumulation studies are unable to assess the equilibrium status of the organisms relative to the contaminants to which they are exposed. Establishing equilibrium provides a reproducible benchmark upon which scientific and management decisions can be based (e.g., comparison to dietary consumption criteria). Unlike bioaccumulating organisms, passive samplers can be assessed for their equilibrium status and are being considered as a surrogate for bioaccumulation at some sites. In this investigation, over a three-year period, we compared the bioaccumulation of selected polychlorinated biphenyls (PCBs) by mussels in water column deployments at the New Bedford Harbor Superfund site (New Bedford, MA, USA) to co-deployed passive samplers. Analysis of the data focused on which approaches for estimating equilibrium bioaccumulation best agreed with calculated passive sampler equilibrium concentrations. In addition, a limited evaluation of metal bioaccumulation by the exposed mussels and a metal passive sampler (i.e., Gellyfish) was performed. In general, mussel and passive sampler accumulation of PCBs was significantly correlated; however, surprisingly, agreement on the magnitude of accumulation was optimal when bioaccumulation and passive sampler uptake were not corrected for non-equilibrium conditions. A subsequent comparison of four approaches for estimating equilibrium mussel bioaccumulation using octanol- and triolein-water partition coefficients (i.e., $K_{OW}$ and $K_{TR}$), and two types of polymer-lipid partition coefficients demonstrated field deployed mussels were not at equilibrium with many PCBs. Interestingly, when combined with equilibrium passive sampler results, the simplest approach using $K_{OW}$ was the best predictor of equilibrium mussel bioaccumulation. This finding suggests this relatively simple approach, along with passive sampling, could be used to estimate equilibrium bioaccumulation during remedial operations at contaminated sites. Bioaccumulation of metals was better correlated with the dissolved metals and not the free metal ions alone. These analyses are intended to assist environmental scientists and managers to interpret their field deployment data when transitioning from biomonitoring to passive sampling.

**4.17.P-Mo135 Optimization of Equilibrium Passive Sampling for Short-Term Surface Water Measurements**

***Oindrila Ghosh*** *and Upal Ghosh, University of Maryland, Baltimore County*

Polymeric passive sampling allows accurate measurement of the freely dissolved concentrations of hydrophobic organic contaminants (HOCs) like polychlorinated biphenyls (PCBs) in water. While significant advances have been made in the development of passive sampling for time-integrated measurements over long periods (months), short-term measurements (days) have not been explored using passive sampling. Short-term surface water measurements are often critical for assessing inputs from episodic events and delineating ongoing sources for which passive sampling is currently not available. In this study we explore the feasibility of designing an equilibrium passive sampler that can perform short-term surface water measurements of PCB concentrations using a combination of mathematical modeling and physical experiments. For the modeling experiments, we used the one-dimensional Fick's diffusion equation to simulate the uptake and loss kinetics of PCB molecules in a polyethylene (PE) sheet from water. Since the water boundary layer (WBL) and the membrane thickness are major rate-limiting zones for mass transfer in passive samplers, we explore the opportunity of manipulating these zones through the design of the sampler and achieve faster equilibration. In order to experimentally verify our modeling results, we designed an artificial flow chamber with a pump controlling the flow within a closed sampling station. PE samplers loaded with a known concentration of performance reference compounds (C13 analogs of PCB compounds) were placed within the sampling station under controlled flow conditions and the fractional loss of the reference compounds tracked. The initial assessments from the conceptual model showed that for a low WBL thickness of 0.01cm (representative of turbulent water conditions) a 1 mil PE was able to come to equilibrium in less than 2 days. We are currently optimizing the choice of sampler designs under a range of water velocities in the laboratory. The optimized passive sampling solution interpreted from the present study will be grounded by the theoretical understanding of time integration in equilibrium passive samplers for short-term surface water measurements.

**4.17.P-Mo136 Interlaboratory study of polymeric samplers for ex situ measurement of freely dissolved hydrophobic organic compounds in sediment porewater**

***Guilherme R. Lotufo**[1], Mandy Michalsen[2], Danny Reible[3], Philip Gschwend[4], Upal Ghosh[5], Alan Kennedy[1], Magdalena Rakowska[3], Adesewa Odetayo[3], John MacFarlane[4], Songjing Yan[5] and Mandar Bokare[5], (1) U.S. Army Corps of Engineers, (2) U.S. Army Engineer Research and Development Center, (3) Texas Tech University, (4) Massachusetts Institute of Technology, (5) University of Maryland, Baltimore County*

This study evaluated the precision and accuracy of multi-laboratory measurements for determining freely dissolved concentrations ($C_{\text{free}}$) of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in sediment porewater (PW) using polydimethylsiloxane (PDMS) and low-density polyethylene (LDPE) polymeric samplers. Four laboratories exposed performance reference compound (PRC) pre-loaded polymers to actively mixed and static *ex situ* sediment for ~ 1 month; two laboratories had longer exposures (i.e., 2 and 3 months). For $C_{\text{free}}$ results, intra-laboratory precision was high for single compounds (CV ≤50%), and for most PAHs and PCBs interlaboratory variability was low (magnitude of difference ≤ factor 2) across polymers and exposure methods. Variability was higher for the highly hydrophobic PAHs and PCBs, which were present at low concentrations and required larger PRC-based corrections, and for naphthalene, likely due to differential volatilization losses between laboratories. Overall, intra- and interlaboratory variability between methods (PDMS vs. LDPE, actively mixed vs. static exposures) was low. Results showed $C_{\text{free}}$ polymer equilibrium was achieved in ~ 1 month during active exposures, suggesting use of PRCs may be avoided for *ex situ* analysis using comparable active exposure; although such *ex situ* testing may not reflect field conditions. Polymer-derived $C_{\text{free}}$ concentrations for most PCBs and PAHs were on average within a factor of 2 compared to concentrations in isolated PW, which were directly measured by one laboratory; difference factors of up to 6 were observed for naphthalene and the most hydrophobic PAHs and PCBs. $C_{\text{free}}$ results were similar for academic and private sector laboratories. Demonstrated accuracy and precision for determination of $C_{\text{free}}$ using polymer sampling is anticipated to increase regulatory acceptance and confidence in method use.

**4.17.P-Mo137 Evaluating the Effectiveness of Select Biomimetic Passive Sampling Methods for Estimating the Toxicity of Petroleum Contamination in Soils**

***Sonja Koster**[1], Allison Rutter[2] and Barb A. Zeeb[1], (1) Royal Military College, Canada, (2) Queen's University, Canada*

The derivation of site-specific remediation guidelines for contaminated sites often requires the use of labour-intensive and time-consuming toxicity tests using indicator organisms. The toxicity of petroleum hydrocarbon (PHC) contamination to soil-dwelling invertebrates as well as PHC uptake in plants has been correlated to the bioaccessible fraction of the hydrocarbon mixtures dissolved in soil porewater. Chemical analytical methods that mimic biological uptake (biomimetic methods) of contaminants from soil pore water can be used to estimate the toxicity to organisms without the need for costly and time-consuming toxicity testing. A selection of biomimetic passive sampling methods, including polyoxymethylene (POM) and solid-phase microextraction (SPME) will be evaluated using contaminated field soils of various concentrations from a site in the Northwest Territories in Canada. The test soils were characterized using standard chemical extraction methods for total PHCs and individual constituents (e.g. parent and alkylated polycyclic aromatic hydrocarbons) as well as total organic carbon, which is known to affect bioaccessibility. The selected passive sampling methods will be used to determine the bioaccessible soil pore water concentrations of total PHCs and individual compounds known to contribute substantially to the toxicity of PHC mixtures. Each passive sampling method will be evaluated based on consistency, time commitment, resource use, and labour requirements, The passive sampling results will also be correlated to concurrent toxicological studies using soil invertebrates and plants from the field site. Preliminary results are discussed.

**4.17.P-Mo138 Sediment Biodegradation: An Alternative Testing Method to OECD 308**
*Thomas Parkerton[1] and **Kelly Marie McFarlin**[2], (1) EnviSci Consulting, (2) ExxonMobil Biomedical Sciences, Inc.*
Quantitative sediment biodegradation rates that mimic those in the natural environment are lacking in literature, as current predictions are based on applying a multiplication factor to water-only test systems. We designed, modeled and validated a novel sediment biodegradation method that incorporated passive dosing to quantify biodegradation rates of hydrocarbons in the upper most layer of freshwater sediment. Natural river sediment was aerated for ~30 days to remove background organic carbon and was combined with freshly collected oxygen-saturated river water at a 1:4 volume ratio in 60 mL amber glass vials. Mesocosms had 20 mL of headspace and were placed on a roller table at 20°C and in the dark for 128 days. Eighteen individual hydrocarbons spanning 5 different chemical classes ($C_6$ – $C_{18}$; log Kow: 3.8 – 7.8) were loaded as a mixture on to silicon rods (1 cm x 1.5 mm). Individual rods were placed in respective treatments and analyzed at 7 time points over 128 days to determine chemical losses over time using GC-FID. Biodegradation was measured by comparing the chemical loss in biotic and abiotic treatments with and without sediment. Environmental analyses included oxygen, Total nitrogen, total phosphorous, orthophosphate, sulfate, iron, and microbial biomass. Sediment was characterized using pH, total organic carbon, dry weight, and grain size. Three Pilot Tests were conducted to determine sorption, oxygen and nutrient limitations. A chemical fate mass balance model was developed based on literature partition coefficients to predict mass transfer kinetics and then validated with empirical data. Preliminary results indicated that sorption kinetics influenced the bioavailability of specific hydrocarbons and their potential to biodegrade in the presence of sediment. This presentation will highlight the measured sediment biodegradation rates, how they compare to model results, and the lessons learned from the Pilot Tests.

**4.18 Transformation of Environmental Contaminants: Reaction Mechanisms and Product Identification**

**4.18.T-01 Hydrothermal degradation of three pharmaceuticals and detection of their transformation products**
***Pierre Oesterle**, Christine Gallampois and Stina Jansson, University of Umeå, Sweden*
In the last 20 years, wastewater treatment plants have been adding to tertiary treatments a polishing step aimed at removing/degrading micropollutants (e.g. pharmaceuticals). Ozonation is often used to degrade such compound however there may be a potential for the creation of more harmful transformation products. Activated carbons can instead be used to adsorb micropollutant on its surface and then be safely discarded or regenerated. Its regeneration often requires a drying step followed by a high thermal treatments (600 °C). To avoid these two steps, we decided to instead use hydrothermal regeneration/recycling as it uses uses water under subcritical conditions and makes it possible to hydrothermally degrade pharmaceuticals concentrated on the spent activated carbon via abiotic processes. From a previous work where we adsorbed ten commonly used/prescribed pharmaceuticals found in wastewater treatment plants in Sweden, we observed that the

temperatures at which the parent compounds were efficiently degraded was above 240°C but we did not account for transformation products. We therefore chose to further study three pharmaceuticals (Trimethoprim, Sulfamethoxazole, Caffein) and characterize its transformation products under such conditions. To be able to detect a wider range of transformation products the samples were both run in suspect screening and non-target screening via LC-MS-QTOF on C18 and HILIC columns. Findings showed that the parent compounds gradually transformed at temperatures above 200 °C and were below limit of quantification at 280 °C. For trimethoprim, more than 10 transformation products were detected compared to 3 and 1 transformation products for sulfamethoxazole and caffeine respectively. A further step in this study will compare the transformation products observed with the ones produced in presence of activated carbons to determine its hazardous potentials.

**4.18.T-02 Jet Plasma Treatment of Poly- and Perfluoroalkyl Substances (PFASs)**

***Moshan Chen**, Trey Oldham, Xiaoshuang Chen, Elijah Thimsen and Kimberly M. Parker, Washington University in St. Louis*

Poly- and perfluoroalkyl substances (PFASs) are widely used anthropogenic surfactants. Due to their persistence, PFASs have been widely detected in natural and engineered water systems including groundwater, surface water, and wastewater effluents. Because PFASs were toxic organic compounds, their presence in water systems threatens the environment and human health. Plasma technology is a promising tool to degrade PFASs in water treatment. Plasma generates reactive oxygen species, reactive nitrogen species, and reactive reductive species such as hydrated electron, which are concentrated at the plasma-water interface. Therefore, the partitioning of surfactant PFASs to the plasma-water interface may primarily contribute to their degradation during plasma treatment. In this study, aim to investigate the role partitioning of PFASs during jet plasma treatment. We first compared the degradation of surfactant PFASs (i.e., perfluorobutanoic acid or PFBA, perfluorooctanoic acid or PFOA) and non-surfactant organic probes (i.e., benzoate, para-hydroxybenzoate) during jet plasma treatment. Using quenchers for reactive species such as hydroxyl radical and hydrated electron, we found that the degradation of organic probes during 20 min plasma treatment was quenched ~50%. However, the degradation of PFBA and PFOA were unchanged by the addition of quenchers, indicating that PFBA and PFOA degradation occurred at the plasma-water interface. Our experiments also showed that PFBA and PFOA degradation was unchanged by the addition of halides, indicating that PFBA and PFOA degradation was independent of water matrices. Regardless of the presence of quenchers and halides, we found that the degradation of PFOA was ~2-fold greater than that of PFBA, likely because PFOA tended to partition more to the plasma-water interface than PFBA. We are currently exploring the degradation efficiency of jet plasma on PFBA and PFOA in competition with other PFAS including perfluorohexanoic acid, perfluoroheptanoic acid, perfluorodecanoic acid, and perfluoroundecanoic acid. Based on our results that PFOA was degraded faster than PFBA, we hypothesize that the degradation of PFASs correlates with their partitioning coefficient from the bulk water to the plasma-water interface. Our study potentially demonstrates that jet plasma, by generating reactive species concentrated at the plasma-water interface, is a robust treatment technology for surfactant PFASs in diverse water matrices.

**4.18.T-03 Concentration and photo-oxidative remediation of 6:2 fluorotelomer carboxylic acid in aqueous aerosol droplets**

*Bailey Bowers, Ryan C. Sullivan and **Riley Madison Weatherholt**, Carnegie Mellon University*

Per- and polyfluorinated substances pose immense challenges to sustainability due to their extreme persistence and toxicity. One unique feature of PFAS is their high surface activity, meaning these compounds are enhanced in the droplet phase relative to bulk solution via aerosolization. Concentration of PFAS into aerosol droplets could be leveraged in remediation strategies to accelerate the kinetics of degradation, creating concentrated droplets from large volumes of water with extremely low levels of PFAS. Photo-oxidative remediation of polyfluorinated compounds such as 6:2 fluorotelomer carboxylic acid (6:2 FTCA) have been successful in the bulk phase, but have yet to be investigated in droplets. We sought to determine if this treatment is feasible in the droplet phase, and determine the rate at which 6:2 FTCA is degraded by ozone and hydroxyl radicals in UV-illuminated aqueous droplets. An Oxidation Flow Reactor was used to subject generated aerosol to intense UV light along with the gas-phase oxidants produced by this photochemistry that include ozone and hydroxyl radical. We observed enhancement factors of 6:2 FTCA in droplets relative to bulk solution of up to 20, which is significantly lower than enhancement factors observed for perfluorinated compounds. Up to 98% of 6:2 FTCA was removed in droplets exposed to UV light and photo-oxidants for 2 minutes. Furthermore, nontarget analysis was performed using ultrahigh resolution Orbitrap mass spectrometry to characterize the transformation products of this aerosol remediation process.

**4.18.T-04 Metabolic markers of AFFF polyfluorinated compound transformation inhibition to microbial biodegradation of aromatic hydrocarbons and chlorinated solvents**

***Christopher Olivares***[1]*, Emily K Cook*[2]*, David L Sedlak*[2] *and Lisa Alvarez-Cohen*[2]*, (1) University of California, Irvine, (2) University of California, Berkeley*

Biostimulation of aromatic hydrocarbons and bioaugmentation of anaerobic dechlorination are commonly applied to remediate co-contaminants in sites impacted by per- and polyfluoroalkyl substances (PFAS). The efficiency of these remediation strategies might decrease if PFAS and other components in aqueous film forming foams (AFFF) inhibit co-contaminant remediation Known PFAS microbial toxicity impacts include stress response and biofilm formation, and they are expected to impact the cell membrane. These insights are based on perfluorinated carboxylates and sulfonates (e.g. PFOA, PFOS) which are negatively charged, but sites impacted by AFFF also include cationic/zwitterionic polyfluorinated compounds and neutral intermediates. In addition,  Their inhibition mechanisms can be confusing because AFFFs are mixtures that can undergo biotransformation; while PFAS can inhibit microbial metabolism, non-fluorinated organics can be used as labile substrates. We are currently studying the impacts of AFFF for two microbial systems: aerobic degradation of aromatic hydrocarbons and anaerobic dehalogenation of trichloroethylene. We monitored changes in degradation rates as well as abundance changes of targeted metabolites with different dilutions of the foam. To study the individual impacts of PFAS found in the foams, we performed individual exposures of polyfluorinated compounds present in foams and compared it to its key transformation products. The vision of this research direction is to understand mechanisms of inhibition, detoxification reactions along the biotransformation pathways, as well as identify key metabolic biomarkers associated with PFAS types.

**4.18.T-05 Abiotic RNA Hydrolysis in the Environment: Implications for the Environmental Fate of Emerging RNA Interference Biopesticides 1 of 2**

***Kimberly M. Parker****, Ke Zhang, Anamika Chatterjee and Kun-Pu Ho Ho, Washington University in St. Louis*

RNA interference (RNAi) is a biological process in which double-stranded (ds)RNA directs the degradation of homologous messenger RNA (mRNA), preventing protein synthesis. In agriculture, several RNAi-based products have been developed using dsRNA as active agents (i.e., dsRNA biopesticides) to protect crops from pests. These agricultural RNAi products include both dsRNA biopesticides generated by dsRNA-expressing genetically modified crops and dsRNA applied exogenously via spray or irrigation water. The ongoing development of dsRNA biopesticides raises the importance of developing a fundamental understanding of the chemical stability of dsRNA molecules, which defines their ultimate potential to persist in biological and environmental systems in the absence of enzymes or microorganisms. This presentation integrates results from a series of studies that evaluated dsRNA abiotic hydrolysis in environmental systems including surface waters, soils, and sediments. In these systems, nucleic acids including dsRNA are distributed between dissolved molecules and molecules adsorbed to various minerals including metal (oxyhydr)oxides. We determined that dissolved single-stranded (ss)RNA undergoes rapid hydrolysis catalyzed by alkaline pH (beyond values typically encountered in environmental systems), as well as at neutral pH by certain metal ions (i.e., lead and copper) that accelerate hydrolysis by acting as Lewis acids. In all cases, we found that dissolved dsRNA was recalcitrant to abiotic hydrolysis likely due to rigidity of its duplex structure. Furthermore, slow intrinsic rates of phosphodiester bond hydrolysis at environmentally relevant pH and metal concentrations regardless of RNA higher-order structure prevent abiotic hydrolysis from contributing to dsRNA degradation in the solution phase. In contrast, we discovered that both ssRNA and dsRNA adsorbed to the surfaces of iron (oxyhydr)oxides undergo rapid hydrolysis on the timescale of hours. Similar to metal ions in solution, iron atoms in the mineral-phase likely act as Lewis acids to catalyze RNA hydrolysis regardless of structure. To our knowledge, this is the only abiotic hydrolysis pathway that occurs at rates competitive with enzymatic hydrolysis of RNA in environmental systems. Coupled with widespread adsorption of nucleic acids to mineral phases in soils and sediments, mineral-catalyzed hydrolysis may be a significant pathway for the degradation of RNAi-based biopesticides in environmental systems.

**4.18.T-06 Abiotic RNA Hydrolysis in the Environment: Implications for the Environmental Fate of Emerging RNA Interference Biopesticides**

***Kimberly M. Parker****, Washington University in St. Louis*

RNA interference (RNAi) is a biological process in which double-stranded (ds)RNA directs the degradation of homologous messenger RNA (mRNA), preventing protein synthesis. In agriculture, several RNAi-based products have been developed using dsRNA as active agents (i.e., dsRNA biopesticides) to protect crops from pests. These agricultural RNAi products include both dsRNA biopesticides generated by dsRNA-expressing genetically modified crops and dsRNA applied exogenously via spray or irrigation water. The ongoing development of dsRNA biopesticides raises the importance of developing a fundamental understanding of the chemical stability of dsRNA molecules, which defines their ultimate potential to persist in biological and environmental systems in the absence of enzymes or microorganisms. This presentation integrates results from a series of studies that evaluated dsRNA abiotic hydrolysis in environmental systems including surface waters, soils, and sediments. In these systems, nucleic acids including dsRNA are distributed between dissolved molecules and molecules adsorbed to various minerals including metal (oxyhydr)oxides. We determined that dissolved single-stranded (ss)RNA undergoes rapid hydrolysis catalyzed by alkaline pH (beyond values typically encountered in environmental systems), as well as at neutral pH by certain metal ions (i.e., lead and copper) that accelerate hydrolysis by acting as Lewis acids. In all cases, we found that dissolved dsRNA was recalcitrant to abiotic hydrolysis likely due to rigidity of its duplex structure. Furthermore, slow intrinsic rates of phosphodiester bond hydrolysis at environmentally relevant pH and metal concentrations regardless of RNA higher-order structure prevent abiotic hydrolysis from contributing to dsRNA degradation in the solution phase. In contrast, we discovered that both ssRNA and dsRNA adsorbed to the surfaces of iron (oxyhydr)oxides undergo rapid hydrolysis on the timescale of hours. Similar to metal ions in solution, iron atoms in the mineral-phase likely act as Lewis acids to catalyze RNA hydrolysis regardless of structure. To our knowledge, this is the only abiotic hydrolysis pathway that occurs at rates competitive with enzymatic hydrolysis of RNA in environmental systems. Coupled with widespread adsorption of nucleic acids to mineral phases in soils and sediments, mineral-catalyzed hydrolysis may be a significant pathway for the degradation of RNAi-based biopesticides in environmental systems.

**4.18.T-07 Physico-Chemical Properties and Environmental Fate Predictions of Dienogest and Its Transformation Products**

*Cassandra Johannessen, Melanie Le and* ***J. Mark Parnis****, Trent University, Canada*

Dienogest is a progestin synthetic steroid hormone derived from testosterone. It is expelled as waste within 24 hours of administration. Dienogest is known to form a variety of steroid transformation products (TPs) that are largely generated through metabolic or photodegradation pathways. Transformation typically proceeds through either hydroxylation, 1,2-dehydrogenation, or hydrogenation. The quantitatively important TPs of dienogest are generally stable in the environment. Select TPs have been shown to be discharged into receiving waters, presenting a potential risk of endocrine toxicity to aquatic biota. Current literature suggests that at least one has more potent antigestagenic and antigonadotropic activity than dienogest, as well as the ability to inhibit pregnancy.

There is little knowledge of their physico-chemical properties of dienogest and its TPs, and corresponding environmental partitioning tendencies and persistence. The objectives of this study were use COSMO-RS solvation theory to estimate environmentally-relevant physico-chemical property data for dienogest and its TPs, and to use this estimated property data to predict with a fugacity-based multimedia model the likely environmental behavior and fate of these compounds. The results indicate that many of the TPs of dienogest have significantly different (orders of magnitude) physico-chemical properties compared with dienogest, and have different partitioning tendencies from one another. For example, the predicted vapor pressure (VP) ranges from 2.43E-16 to 6.33E-08 for the TPs studied, with dienogest having an intermediate VP of 6.14E-12. Similarly, the water solubility ranges from 0.43 to 2,700 mg/L with dienogest again having an intermediate predicted solubility of 437 mg/L.

The environmental distribution and persistence of dienogest, as estimated by Level III EQC modeling, substantially depends on its route of entry into the environment. For example, if emitted into water only, it is expected that almost all of the dienogest will remain in water and have an environmental residence time of 10 hours. By contrast, if the dienogest is applied to soil, 86% of the dienogest is expected to be distributed in soil while the rest is expected to partition to water. The estimated environmental behavior and fate of dienogest and its TP is not predicted to substantially differ from one another even though significant alterations to the core structure of dienogest take place during transformation.

**4.18.T-08 Impact of Reactive Oxygen Species Scavenging on the Intermediate Production of Anthracene and Anthraquinone in Fresh vs Saltwater Environments**

***Scott St. Romain**, Kevin Armbrust and Laura M. Basirico, Louisiana State University*

Hydrophobic organic contaminants (HOCs) are frequently detected in estuarine environments where the chemical composition of water varies particularly with respect to salinity. While salinity demonstrates the ability to substantially alter HOC degradation pathways, only few experiments account for the complex composition of natural waters, including salinity, on the degradation and intermediate production of HOCs. Since HOC photoproducts are often more toxic than their parent compounds, accurate characterization of HOC photoproducts in estuarine environments is needed to predict their health effects. Moreover, HOC degradation by hydroxyl radicals produced in natural waters is frequently cited as an important dissipation mechanism, yet its significance on intermediate dissipation is rarely assessed. Consequently, the influence of salinity on the product formation of anthracene and anthraquinone remains poorly characterized despite frequently being detected in saline waters. Furthermore, the reactivity of hydroxyl radical with anthracene's intermediates, anthraquinone, anthrone, and 1-hydroxyanthraquinone, remains uncharacterized. This study elucidates the role of reactive oxygen species (ROS) scavenging by halides in seawater on the formation of anthraquinone during the photolysis of anthracene in addition to 1-hydroxyanthraquinone and anthrone during the photolysis of anthraquinone. Furthermore, the reactivity of hydroxyl radical with anthraquinone, 1-hydroxyanthraquinone, and anthrone in aqueous environments will be evaluated. Preliminary results show salinity enhances the persistence of anthraquinone during the photolysis of anthracene by a factor greater than four in comparison to deionized water. In addition, salinity suppresses the formation of 1-hydroxyanthraquinone but promotes the formation of anthrone during the photolysis of anthraquinone. This research illustrates the importance of considering the influence of ROS scavenging by halides on organic contaminant degradation in seawater and describes the reactivity of hydroxyl radical with reactive intermediates to better understand their fate in natural waters.

**4.18.P Transformation of Environmental Contaminants: Reaction Mechanisms and Product Identification**

**4.18.P-Mo139 Ozonation of phenol in the presence of biochar and carbonaceous materials: The effect of surface functional groups and graphitic structure on the formation of reactive oxygen species**

***Seok-Young Oh** and Thi-Hai Anh Nguyen, University of Ulsan, Korea, Republic of (South)*
To develop a novel oxidation process using ozone and carbonaceous materials for water treatment and groundwater remediation, the effect of carbonaceous materials on the ozonation of phenol was investigated via batch experiments. Anode carbonaceous material (ACM) recovered from spent Li-ion batteries, graphite, granular activated carbon (GAC), and rice straw-derived biochar were evaluated as catalysts to enhance the ozonation of phenol. Compared with direct ozonation (28% removal in 2 h), the addition of carbonaceous materials enhanced the oxidation of phenol by ozone, showing 47%, 45%, 81%, and 74% removal with ACM, graphite, GAC, and biochar, respectively. According to the quenching experiments with chemical reagents and electron paramagnetic resonance analysis, reactive oxygen species, such as hydroxyl radicals (×OH), superoxide radicals ($O_2^{\times-}$), and singlet oxygen ($^1O_2$), as well as electron transfer in the mediated-reaction, are included in the ozonation of phenol in the presence of carbonaceous materials. The oxygen-containing surface functional groups and graphitic structure are responsible for the reactive oxygen species and electron transfer reactions, respectively. Our results suggest that carbonaceous materials may be effectively used as catalysts to promote the ozonation of recalcitrant contaminants in water.

### 4.18.P-Mo140 Interactions of RNA Biopesticides and Organic Matter in Environmental Systems

***Anamika Chatterjee**, Ke Zhang and Kimberly M. Parker, Washington University in St. Louis*
Novel RNA interference (RNAi) biopesticides applied to control crop pests lead to the release of double-stranded (ds)RNA to the environment. To assess the potential ecological risks associated with their release, a thorough evaluation of dsRNA environmental fate is crucial in receiving soil and aqueous environments. In these environments, dsRNA can bind to various environmental components. In particular, dsRNA has been demonstrated to bind to soil particles and mineral surfaces. Additionally, DNA has been proposed to bind to organic matter (OM). Binding of dsRNA to OM will behave similarly to DNA because both DNA and dsRNA exhibit a phosphodiester backbone. In the case of DNA, binding to OM imparted protection from nuclease-mediated biotic degradation. Since OM is ubiquitous in natural environments, assessing dsRNA binding to OM and the consequent protection from biotic degradation will improve the assessment of dsRNA fate. Therefore, we evaluated the effect of various OM on RNAse I-mediated dsRNA degradation by quantifying the production of the 3'-adenosine monophosphate (3'-AMP) end product. We found that dsRNA incubated with RNAse I in the presence of 10 mgC/L Pahokee Peat or Leonardite humic acid did not produce 3'-AMP, indicating that humic acids can protect dsRNA from biotic degradation. We characterized the binding interactions between humic acids and dsRNA via a novel gel electrophoresis-based binding assay and we determined that this protective effect is, in part, due to the strong binding between dsRNA and humic acids. In contrast to humic acids, 10 mgC/L Suwanee River or Elliott Soil fulvic acid inhibited the production of 3'-AMP by 67 and 47%, respectively. It is probable that fulvic acids do not protect dsRNA from nuclease degradation as well as humic acids due to their inability to bind dsRNA. While binding of released dsRNA is likely to reduce its transport and bioavailability, binding provides protective effects against nuclease-mediated degradation leading to dsRNA persistence.

### 4.18.P-Mo141 Production of Dichloroacetonitrile from Derivatives of Isoxaflutole Herbicide during Water Treatment

***Jacqueline Rogers**, Moshan Chen, Kaichao Yang, Jonathan Graham and Kimberly M. Parker, Washington University in St. Louis*
The phytotoxic herbicide isoxaflutole has been increasingly used in recent years. Potential increases of isoxaflutole use in the coming years may result due to the release of genetically modified (GM) isoxaflutole-tolerant soybeans in 2020. Isoxaflutole is rapidly converted to diketonitrile, which is the herbicidally active and environmentally dominant form. Diketonitrile can negatively impact drinking water quality as a contaminant itself. However, diketonitrile also presents a risk to drinking water quality due to its transformation during disinfection. Diketonitrile is a known disinfection-by-product (DBP) precursor for dichloroacetonitrile (DCAN), an unregulated but highly toxic nitrogenous DBP. In this study, we quantified DCAN formation from

diketonitrile in the presence of both free chlorine and chloramine for the first time to determine the risk of diketonitrile in drinking water sources. We found diketonitrile produces DCAN at circumneutral pH within seconds with free chlorine and over hours with chloramine at approximately 100% yields. At such high yields, diketonitrile produces DCAN 10 to 100-fold higher than other known precursors. Diketonitrile degradation rates decreased from neutral pH to acidic and basic pH with free chlorine, resulting in maximum reaction rates at neutral pH. In comparison, diketonitrile degradation rates with chloramine were $10^5$-fold slower than with free chlorine and decreased from acidic to basic pH. Based on our data, we propose a reaction pathway for diketonitrile degradation by free chlorine via base-catalyzed halogenation by hypochlorous acid and subsequent nucleophilic attack at the ketones of diketonitrile. We also propose the first pathway for diketonitrile degradation by chloramine with a similar pathway that occurs via acid-catalyzed halogenation by monochloramine and the same subsequent nucleophilic attack. Our results indicate increasing use of isoxaflutole will result in greater occurrences of diketonitrile, which is a high-yield DCAN precursor during disinfection.

**4.18.P-Mo142 Photodegradation of Double-Stranded RNA in Solution and on Surfaces: Mechanisms for Fast Degradation of RNA Interference Biopesticides on Surfaces**

***Kun-Pu Ho Ho**, Megan Ross, Ke Zhang and Kimberly M. Parker, Washington University in St. Louis*

The application of double-stranded RNA (dsRNA) biopesticides is an emerging crop protection technology that employs RNA interference (RNAi) to degrade homologous messenger RNA (mRNA), impeding the synthesis of essential proteins in pest species. Recently, several studies have developed foliar-applied dsRNA biopesticides against an extensive array of main insect pests; however, to be effective, the stability of these designed biopesticides upon application to leaf surfaces must be sufficient under exposure to sunlight. Furthermore, dsRNA biopesticides released to receiving aqueous environments may undergo both direct and indirect photodegradation to be removed from the environment. In this study, we investigated the sunlight photolysis rates and mechanisms of dsRNA in solution and on surfaces. In solution, we examined dsRNA direct photolysis in phosphate buffer and indirect photodegradation by adding a photosensitizer (i.e., nitrate, dissolved organic matter, DOM). After 16.5 h irradiation 12.7±0.3% of the dsRNA was degraded by direct photolysis, corresponding to a degradation rate constant of $8.22(\pm0.70) \times 10^{-3}$ hour$^{-1}$ assuming first-order kinetics. In comparison, the photodegradations in the presence of DOM and nitrate were 45.5±0.3% and 49.2±1.7%, respectively, indicating indirect photodegradation dominates. Next, we investigated the direct photolysis of dsRNA on a hydrophilic surface (i.e., glass) and a hydrophobic surface (i.e., polytetrafluoroethylene, PTFE). Over 24 h irradiation, the direct photolysis of dsRNA on the glass surface followed first-order kinetics with a rate constant of 0.11±0.01 hour$^{-1}$. We found that dsRNA degradation on glass was 4-fold higher than degradation on PTFE and 13-fold higher than direct photolysis in solution; and try to find possible reasons causing the faster degradation from reactor irradiance, light absorption of dsRNA, and product determination in the next steps. The fast direct photolysis of dsRNA on surfaces implies that this photodegradation mechanism may have an important role in naturally attenuating foliar-applied dsRNA biopesticides. Furthermore, both direct photolysis on surfaces and solution-phase photodegradation (particularly indirect) may be important for assessing the environmental fate of dsRNA biopesticides regardless of application method.

**4.18.P-Mo143 COSMOtherm Estimates of Organophosphate Ester Oxidation Product Physico-Chemical Properties**

*Cassandra Johannessen and **J. Mark Parnis**, Trent University, Canada*

Organophosphate flame retardants (OPFRs) are synthetic, high-production volume chemicals of emerging concern. TCPP (Tris (2-chloroisopropyl) phosphate) and EHDP (2-ethylhexyl diphenyl phosphate) are two of the most dominate organophosphate ester flame retardants present in air. Both compounds are suspected to pose a high risk to the environment, and TCPP is cited as a potential carcinogen. TCPP is a representative chlorinated OPFR, while EHDP is a representative non-chlorinated OPFR with both phenyl and alkyl groups. OPFRs with low volatility are mostly associated with environmental occurrence in airborne particles. Thereby, their chemical fates are likely dictated by heterogeneous oxidation.

The heterogeneous OH oxidation of particulate TCPP and EHDP have been previously investigated. Transformation products (TPs) and their mechanism of formation have been proposed for both TCPP and EHDP. Notably, the TPs of the above OPFRs are estimated to pose equal or greater risk to aquatic and terrestrial health than their parent compounds. Although TPs of TCPP have been detected globally in elevated amounts and it is suspected that TPs of EHDP are ubiquitous as well, there is limited understanding of the partitioning tendencies of these compounds. Thus, it remains unclear how atmospheric OH and NOx mediated transformation impacts the environmental fate of both chlorinated and non-chlorinated OPFRs.

The primary objectives of this study were to predict physico-chemical partitioning properties of two model OPFRs and gain a preliminary understanding of how the environmental fate of both chlorinated and non-chlorinated airborne OPFRs are altered upon heterogenous transformation. As well, a second objective was to compare two common property prediction methods, COSMOtherm and OPERA, in terms of their impact on environmental fate assessments for the two suites of OPFR transformation products. The results show that heterogeneous oxidation has a significant impact on the partitioning behaviour of the various oxidation products. There is a general decrease in log Kaw with increasing extent of oxidation, leading to a lowering in vapour pressure and increased distribution to aqueous (TCCP) and lipid-rich (EHDP) media. As well, the agreement between the two methods is shown to be best for chemicals that are within the applicability domain of OPERA.

**4.18.P-Mo144 The Effect of Flame Retardants on Toxics Emitted from Foam Combustion**

*Bailey Bowers[1], Amila O. De Silva[2], Heather M. Stapleton[3], Ryan C. Sullivan[1] and **Riley Madison Weatherholt**[1], (1) Carnegie Mellon University, (2) Environment and Climate Change Canada, (3) Duke University*

To meet flammability standards initially introduced in the 1970s, furniture manufacturers often add chemical flame retardant mixtures to furniture foams. Flame retardants have been demonstrated to have a wide range of toxic effects in and of themselves, but very little research has been conducted on the effects these flame retardants have on the combustion process and resulting emissions. Since flame retardants work by making combustion less efficient, we hypothesize that foams treated with flame retardants will yield higher amounts of incomplete combustion products than foam without flame retardants. Furthermore, incomplete combustion products are more toxic than the products of complete combustion, meaning that flame retardants could actually be making combustion emissions more toxic. It has been previously demonstrated by others that this hypothesis holds true with regard to production of carbon monoxide and hydrogen cyanide, but characterization and comparison of persistent organic pollutants and novel transformation products emitted from foams with and without flame retardants has not been conducted. We combusted foams with and without flame retardants in a steel chamber, collected the emissions on quartz filters and sorbent tubes, and used gas chromatography-mass spectrometry (GC-MS) and ultra performance liquid chromatography coupled to high resolution Orbitrap mass spectrometry (UPLC-HRMS) to characterize the emissions. We find that combustion of FR-laden foams releases intact flame retardant molecules, such as tris(2-chloroisopropyl)phosphate and 2-ethylhexyl 2,3,4,5-tetrabromobenzoate. While it is generally thought that organophosphate flame retardants stay within or on the surface of the foam, polymerizing to create a char layer that suppresses combustion, we observe that up to 4% of the FR mass in the foam is released during combustion. Furthermore, toxic halogenated transformation products of flame retardants were also measured. Our work suggests the emission of intact flame retardants and halogenated byproducts should be considered when evaluating the benefits versus hazards of adding flame retardants to furniture foam and other consumer products.

**4.18.P-Mo145 Hepatic Biotransformation of N-(1,3-Dimethylbutyl)-N'-Phenyl-P-Phenylenediamine-Quinone (6PPD-Q) in Rainbow Trout, an Acutely Sensitive Species.**

***David James Montgomery**, Markus Brinkmann, Markus Hecker, Xiaowen Ji, Matthew Schultz, Alper James Alcaraz and Summer Selinger, University of Saskatchewan, Canada*

6PPD-Q is an abiotic transformation product of 6PPD, a tire anti-degradant. 6PPD-Q is deposited onto roads and then dispensed into waterways through stormwater and snowmelt. 6PPD-Q is acutely toxic (24 hours) to coho salmon, brook trout and rainbow trout but tolerated by many other fishes including white sturgeon and Artic char. However, little is known about its mechanisms of toxicity and whether the culprit is the parent chemical or a metabolite. Therefore, it is important to determine if 6PPD-Q is biotransformed to potentially explain its highly species-specific toxicity. In this study, an isolated liver perfusion assay was used to quantify the biotransformation of 6PPD-Q and determine associated toxicokinetic parameters in rainbow trout. This assay measured the hepatic extraction fraction (E) used to calculate clearance (Cl) of 6PPD-Q. An active transport inhibitor, Cyclosporin A (CsA), was introduced midway throughout the assay to assess active transport from plasma into hepatocytes. Furthermore, to determine the specific metabolites formed *in vivo*, bile samples from rainbow trout acutely exposed (< 96 hours) to 6PPD-Q were analyzed utilizing liquid chromatography-mass spectrometry (LCMS). The results revealed a high E (> 0.70), no difference in E or Cl values with CsA administration, and discovery of an oxy-glucuronide metabolite with a hydroxy-fragment in rainbow trout bile. Further research will focus on tolerant species bile analysis in addition to *in vitro* hepatocyte and gill biotransformation assays.

## 4.18.V Transformation of Environmental Contaminants: Reaction Mechanisms and Product Identification

**4.18.V-01 Removal and Mineralization of Nitrotriazolone in Contrasting Freshwater Sediment Systems Using 13C and 15N Stable Isotope Tracers**

***Thivanka S. Ariyarathna** and Craig Tobias, University of Connecticut*

Environmental fate of insensitive high explosive compound nitrotriazolone (NTO) has not been adequately identified in surface freshwater environments although it is of great concern due to its high polarity and associated higher potential for offsite migration. The goal of this study is to evaluate and quantify removal and mineralization of NTO in freshwater sediment systems under natural and bio-stimulated conditions. We conducted aquaria-scale experiments using three geochemically and texturally contrasting freshwater sediment types: low organic carbon (OC) river sand, high OC wetland sediment, high OC pond silt with and without submerged macroalgae to investigate natural attenuation of NTO. Similarly aquaria-scale bio-stimulation experiments were also conducted using high OC pond silt mixed with two types of organic matter: leaf litter (high C:N) and algae (low C:N) to compare the differences in NTO processing rates and pathways with natural attenuation. 50 L aquaria maintaining oxic water column and hypoxic/anoxic sediment layer were spiked with isotopically labelled ($^{13}C$ and $^{15}N$) NTO as a single pulse input with a target concentration of 1.5 mg $L^{-1}$. Experiments were conducted over three weeks, and time series aqueous samples and sediment samples were collected. Dissolved NTO and inorganic mineralization products including $^{15}NO_{2,3}^-$, $^{15}NH_4^+$, $^{15}N_2O$, $^{15}N_2$, and $^{13}C$-DIC (dissolved inorganic carbon) and total $^{13}C$ and $^{15}N$ isotopic compositions of solids in the aquaria were quantified. Adsorption of NTO or its derivatives onto solids was not a significant path of environmental fate of NTO. However, mineralization of NTO was seen in all the treatments and amount of mineralization products formed decreased from wetland > pond silt + submerged macrophyta > pond silt > river sand treatments under natural environmental conditions. We observed the highest rates of NTO loss and mineralization in the organic matter addition experiments compared to all other natural treatments. Two isotope tracers, $^{13}C$ and $^{15}N$ based total mineralization kinetics of NTO in each of these treatments will be modelled separately and validated each other to get a robust estimate for NTO mineralization. The results of this study will propose mineralization pathways, identify possible factors affecting mineralization and therefore have the potential to aid in parameterization of fate and transport models and contaminant management schemes.

## 4.19 Using Measured Data of Chemicals in Environmental Risk Assessment: Improving the Understanding of Uncertainties

### 4.19.T-01 Introductory Remarks: Using measured data of chemicals in environmental risk assessment

***Graham Merrington***[1] *and Lisa Nowell*[2]*, (1) wca environment limited, United Kingdom, (2) U.S. Geological Survey*

The May 2022 SETAC Technical Workshop on the Criteria for Reporting and Evaluating Exposure Datasets (CREED) brought together experts on the collection, synthesis, and use of measured and modelled chemicals data in environmental matrices. The goals of the workshop were to review the available literature for best practices and guidelines regarding exposure data usability and applicability and develop a common anthology for the pragmatic use and evidence-based use of these data; identify reliability and relevancy criteria for evaluating environmental concentration data; and, propose approaches for summarizing data to facilitate 'fit for purpose' usage. Currently the outputs from this workshop are being tested by experts from over 30 countries to assess the criteria for reliability and relevance across measured environmental data regarding the application to three specific purposes (or uses). This introductory presentation will provide the background and overview of the challenges associated with obtaining consistent, reliable and relevant measured chemical concentrations of chemicals for exposure data input into ecological risk assessments and set the stage for the presentations in the session and the concluding discussion.

### 4.19.T-02 Is it necessary to evaluate the reliability and relevance of measured chemical data in the environment?

***Graham Merrington***[1]*, Lisa Nowell*[2] *and Chuck Peck*[3]*, (1) wca environment limited, United Kingdom, (2) U.S. Geological Survey, (3) U.S. Environmental Protection Agency*

Measurements of chemicals in the environment are used for a wide range of purposes, from simple environmental status reporting to legally binding compliance monitoring and complex risk assessments. Compared with chemical ecotoxicity data, for which prescriptive frameworks of evaluation exist in many regulatory jurisdictions, these measured data, often termed chemical exposure data, generally receive only cursory assessment by practitioners. The evaluation of exposure datasets is not immune to the potential inconsistencies of expert judgments, introducing bias and bad practice, and leading to the drawing of spurious conclusions. Exposure data should be evaluated for both reliability and relevance. Assessment of reliability refers to the inherent quality of the dataset, and evaluates the methods for sample collection, chemical analysis, and data processing steps. An understanding of the sampling design by which data were originally collected can help inform data processing decisions.

We recognise chemical exposure data requirements will be different for different end uses or purposes. Chemical exposure data relevancy assessment evaluates whether the dataset in question is appropriate for a specific end use or application; can it provide useful information for answering the questions that are posed by that application? This assessment typically considers the study design by which the data were originally collected and processed to establish what landscape and conditions are represented by the dataset and whether the dataset is appropriate for the evaluator's purpose.

This work is part of a SETAC Technical Workshop[1] from which the outcomes will be published at the end of 2022. Specifically, these outcomes, described in platform presentations within this same session, provide 'best practice' for the evaluation of exposure data reliability and relevance. This work is also useful to the authors of chemical monitoring studies and the maintainers of publicly available monitoring datasets, as it addresses information that needs to be provided to ensure chemical datasets will be fit for use in exposure and risk assessment.

Examples of the necessity of this assessment will be given using existing datasets and how regulatory decision making have been changed, if this best practice had been followed.

[1] Using Environmental Concentration Exposure Datasets in Environmental Assessments: The Development of Criteria for Reporting and Evaluating Exposure Datasets (CREED).

**4.19.T-03 Evaluating the Reliability of Environmental Concentration Data to Characterize Exposure for Use in Environmental Risk Assessments**

***Michelle L. Hladik***[1]*, Arjen Markus*[2]*, Heinz Ruedel*[3]*, Drew Szabo*[4]*, Iain A. Wilson*[5]*, Stefano Polesello*[6]*, Lisa Nowell*[1] *and Bradley Clarke*[7]*, (1) U.S. Geological Survey, (2) Deltares, Netherlands, (3) Fraunhofer Institute for Molecular Biology and Applied Ecology, Germany, (4) Stockholm University, Sweden, (5) wca environment limited, United Kingdom, (6) Water Research Institute - Italian National Research Council IRSA-CNR, (7) The University of Melbourne, Australia*

Environmental risk assessments often rely on measured concentrations in environmental matrices to characterize exposure of the population of interest—typically, humans, aquatic biota, or other wildlife. Yet there is limited guidance available on how to report and evaluate exposure datasets for reliability and relevance, despite their importance to regulatory decision-making. As part of the SETAC workshop held in May 2022 on "Environmental Concentration Exposure Datasets in Environmental Assessments: The Development of Criteria for Reporting and Evaluating Exposure Datasets (CREED)", specific criteria have been identified to systematically evaluate the reliability and relevance of environmental exposure datasets. Relevance criteria are presented elsewhere in this session. Reliability criteria can help data users understand and characterize uncertainties when existing data are used in risk assessments and can serve as guidance on best practice for the reporting of data for data generators (to maximize utility of their datasets to risk assessors). These items include "gateway" criteria that are minimum requirements and additional criteria used to evaluate reliability in more depth. Although most reliability criteria are universal, some practices may need to be evaluated considering the purpose of the risk assessment. Reliability criteria address univocal identification of variables and study sites, environmental matrices, sampling date, data management, sample collection methods, analytical method performance, data handling/aggregation, treatment of censored data, and generation of summary statistics. Criteria include a scheme for scoring datasets in each of these categories and can be used in conjunction with relevancy criteria (assessed separately as part of CREED) to determine the extent to which environmental monitoring datasets are "fit for purpose," i.e., suitable for use in various types of risk assessment.

**4.19.T-04 Criteria for Evaluating the Relevance of Environmental Exposure Datasets**

***Adam Peters***[1]*, Claus Svendsen*[2]*, Stephen Lofts*[2]*, Michael Beking*[3]*, Andrew J. Harford*[4]*, Michael Hamer*[5]*, Derek Wallace*[6]*, Leonard Oste*[7]*, Rochelle Bohaty*[8]*, Thomas Backhaus*[9] *and Jeanne Vuaille*[10]*, (1) wca environment limited, United Kingdom, (2) UK Centre for Ecology & Hydrology (UKCEH), (3) Environment and Climate Change Canada, (4) Department of Agriculture, Water and the Environment, Eaton, Australia, (5) Syngenta, United Kingdom, (6) ERM Regulatory Services Limited, United Kingdom, (7) Deltares, Netherlands, (8) U.S. Environmental Protection Agency, (9) University of Gothenburg, Sweden, (10) EEA*

Evaluation schemes have been in place for the assessment of ecotoxicological data for quite some time, such as the Klimisch and CRED scoring systems. These systems help to ensure that the hazard data that are used in environmental assessments are both reliable and relevant for any particular application. However, comparable approaches for the evaluation of environmental exposure datasets have been much more limited, and those that are available have tended to focus predominantly on the analytical aspects of the datasets, i.e. a focus on some aspects of reliability but not relevance. Environmental monitoring data are often used for purposes other than those that they were originally intended for, and it is important that the appropriateness for any assessment purpose can be evaluated objectively. Equally, it is important to recognise that perfect datasets for some purposes may never exist, and there will be occasions where the best available dataset has a significant number of limitations for the required application. Therefore, a structured mechanism for evaluating the dataset and clearly identifying any important limitations with respect to its use is highly desirable. This presentation provides guidance on the recommended approach for evaluating the relevance of environmental exposure datasets when compared to the purpose of the assessment. An especially important issue is that the purpose of the assessment or application needs to be clearly defined at the start of the evaluation process. This is because the relevance of the dataset will be different for different applications and purposes. For example, the requirements of a dataset for risk assessment may be similar to those for a statutory compliance assessment but are likely to be different to those for trend analysis or model validation purposes.

**4.19.T-05 Toward Practice Implementation of the CREED Approach for Environmental Assessments**
***Adam C. Ryan***[1]*, Carolina Di Paolo*[2]*, Irene Bramke*[3]*, Ryan R. Otter*[4]*, Jennifer Stauber*[5]*, Yves Verhaegen*[6]*, Zhanyun Wang*[7] *and Caroline Whalley*[8]*, (1) International Zinc Association, (2) The Dow Chemical Company, (3) AstraZeneca BV, Den Haag, Netherlands, (4) Middle Tennessee State University, (5)CSIRO, Australia, (6) CONCAWE, Belgium, (7) ETH Zürich, Switzerland, (8) European Environment Association*
As part of a multiday SETAC Technical Workshop held prior to the SETAC Europe meeting in Copenhagen, Denmark, a group of environmental risk assessment practitioners developed Criteria for Reporting and Evaluating Exposure Datasets (CREED). The goal of the CREED is to improve the transparency and consistency through which exposure data are evaluated for usability in environmental assessments. Recently, CRED (Criteria for Reporting and evaluating Ecotoxicity Data) accomplished the same goal for evaluating potential use of effects data (i.e., to characterize potential hazard) in environmental risk assessments.

This presentation will summarize the synthesis of the CREED process, how to interpret the outcome of the CREED data usability evaluation, and then it will discuss results of a beta test of the CREED approach. Because the approach is currently under development, a beta testing component (applying the CREED approach to multiple exposure datasets for various defined purposes) is viewed as instrumental. The objective of the beta testing is threefold: to evaluate the consistency of scoring among a tripartite pool of beta testers, to receive feedback regarding beta tester disposition toward the approach, and to receive feedback that can be used to refine the CREED approach. Both quantitative and qualitative aspects of the beta test will be discussed. In addition to the beta testing results, a prototype dataset report card will be demonstrated to communicate the outcome of the CREED data usability evaluation and to summarize important attributes of the dataset. It is anticipated that the dataset report card will provide a concise reference pertaining to the usability of a given dataset for a specified purpose.

The CREED approach is not meant to be a replacement for expert judgement, but it is meant to provide a framework by which expert judgement is documented so that data use decisions are both transparent and consistent. The approach is meant to be applicable across jurisdictions and for a variety of user-defined purposes. In that sense, CREED is intended to be a harmonized set of “best practices” for risk assessment practitioners.

**4.19.T-06 A meta-analysis of the occurrence of alkylphenols and their ethoxylates in surface waters and sediments in the United States**
***John L. Newsted***[1]*, Dusty Tazelaar*[1]*, Lauren A. Kristofco*[2] *and Barbara Losey*[3]*, (1) Ramboll, (2) The Dow Chemical Company, (3) Regulatory Network, Inc.*
Nonylphenol (NP), octylphenol (OP), and their ethoxylates (NPEO and OPEO) have been the subject of considerable scientific and regulatory attention, primarily due to concerns about their aquatic toxicity and endocrine activity. Environmental monitoring has been conducted for these substances in the U.S. for several decades. This presentation develops an updated, statistically based meta-analysis of the occurrence and ecological relevance of these substances in fresh and marine surface waters and sediments in the U.S. between 2010 and 2020. Within this dataset a large proportion (76-100%) of all NP, NPEO, OP and OPEO samples were below their respective method Limit of Detection /Limit of Quantification (LOD/LOQ). The objectives were:

(1) to evaluate the impact of analytical detection limits (DLs) and treatment of non-detect (NP) data on reported results, (2) to summarize and evaluate recent (2010-2020) occurrence and concentrations of these substances in surface waters and sediments in the U.S., (3) to conduct a screening risk assessment of these substances to aquatic organisms in U.S. surface waters and sediments, and (4) examine uncertainties related to trend analysis using spatially and temporally inconsistent datasets with variable analytical detection capabilities between studies. A comparison of NP surface water averages (all data vs detects only) within a given year demonstrated that ND proxy values drove the reported average concentrations indicating that ND selection can significantly impact the uncertainties associated with assessing the occurrence and potential ecological significance of NP/NPEOs and OP/OPEOs in water and sediments. Given the preponderance of NDs for these compounds proxy values were imputed using robust regression of order statistics (ROS). Even considering various uncertainties, a screening environmental risk assessment based on ROS treatment of NDs indicated that less than 1% of all samples exceeded the U.S. or Canadian environmental quality guidelines with no exceedances being noted after 2016, indicating a low potential for risk to aquatic organisms.

**4.19.T-07 Measured Pollen and Nectar Residues in Semi-Field Studies to Assess Risk to Pollinators**
***Stefan Kroder****, Seamus Taylor, Gali Nisenboim, Keren Carmi and Miriam Frugis, ADAMA Agricultural Solutions*
The risk of plant protection products to pollinators is majorly driven by contaminated pollen and nectar fed by the adults and possibly carried into the nest as provision to the offspring. The bees are probably the most relevant pollinator group in agricultural landscapes, and the risk assessment tool BeeREX by the United States Environmental Protection Agency as well as the excel bee tool of the European Food Safety Authority use estimates of residue unit doses that represent worst-case scenarios based on historical data bases. These can be replaced by empiric residue measurements in (semi-)field studies reflecting substance-specific dynamics of chemicals on their way from the plant to the different bee life stages. The foraging adults are directly exposed to contaminants on the flowers while larval stages are indirectly exposed by the pollen and nectar carried into the nests or hives and mixed into their diets. In addition to substance-specific properties, exposure levels can vary depending on application method and timing, attractiveness of treated crops and among different pollinator species. ADAMA conducted a number of studies in tunnels and in the field to measure residue levels on pollen and nectar from various crops like oil-seed rape, strawberry, apple, cotton, alfalfa and *Phacelia tanacetifolia* in the United States and in Europe. The sampling methods and regime were adapted to exposure routes of adult and larval bees revealing the residue dynamics within the days after application, and the application schemes took into account specific use patterns of the test items. The results of residues from pollen and nectar hand-collected directly from flowers revealed a much greater variance than pollen and nectar residues sampled from forager bees. While residues of pollen and nectar carried into the hive by forager bees can be assumed to be the most realistic exposure level to younger life stages it may be a case-by-case evaluation whether the flower residue levels are more relevant to older adult bees. Further trials were also performed by using sodium chloride as generic tracer item to compare the levels of contaminants on forager bees when pollen and nectar were collected from different plants. The results show the potential of increasing realism within the pollinator risk assessment and allow different approaches of integrating the empiric residue values combined with consumption estimates into the pollinator risk assessments.

**4.19.T-08 Discussion: Using measured data of chemicals in environmental risk assessment**
***Graham Merrington**[1] and Lisa Nowell[2], (1) wca environment limited, United Kingdom, (2) U.S. Geological Survey*
The reliability of chemical exposure data refers to the inherent quality of the dataset. In contrast, the relevance of chemical exposure data addresses the suitability of the data for an intended purpose of use or assessment.

An understanding of the objectives and study design with which a dataset is, or was, originally collected is needed to determine what conditions the samples in that dataset represent. Following the presentations, we'll undertake to discuss these topics, and their application in the real world. For example: What elements of a chemical monitoring study are critical to demonstrating data reliability? What elements are critical to ascertaining whether data are relevant for a given assessment purpose? If certain information is missing, should this limit the use of the dataset and if so, how? When merging datasets for a given assessment purpose, for example from different geographical areas, what parameters need to be evaluated for compatibility? If using data from a secondary (pre-aggregated) dataset for a given assessment purpose, how important is it to obtain information about the design and methods for the original (component) studies and is it necessary to go back to the original data? There is a recognized understanding of many of the challenges, here we look to focus the discussion on identification of ways forward on the journey to develop consistent criteria for reporting and evaluating chemical exposure data.

**4.19.P Using Measured Data of Chemicals in Environmental Risk Assessment: Improving the Understanding of Uncertainties**

**4.19.P-Th086 Consequences of Variation in Extraction Technique: Fine Particulate Matter (PM2.5) Filters Collected in Arkansas**

***Amelia Margaret Craze***, *Christopher T. Bartle and Courtney Roper, University of Mississippi*

Nearly ninety-nine percent of the global population is breathing polluted air. Fine particulate matter ($PM_{2.5}$) is a criteria air pollutant and exists as a complex mixture of solid and liquid air pollutants that are 2.5 microns or smaller. Linked to a variety of adverse health impacts with various biological mechanisms, $PM_{2.5}$ has been hypothesized to induce oxidative stress. Due to federally mandated air quality monitoring, state environmental agencies monitor $PM_{2.5}$ levels, usually by deploying samplers to collect size-selective particles onto filters. Prior to toxicological and chemical analysis, particles must be removed from filters. However, there is not standardization in filter extraction techniques, creating the potential for methods biases. Recent studies have demonstrated that differences in filter extraction methods cause variable toxicity responses. The objective of this study was to extract evenly divided quadrants of $PM_{2.5}$ filters in different solvents to determine if the extraction method used impacts the chemical composition and oxidative potential of the samples. $PM_{2.5}$ filters were collected by the Arkansas Department of Environmental Quality at four locations during the same 24-hour collection periods, across seasons during 2012 (n=60). Black carbon data was collected prior to splitting each filter into quadrants. Filter quadrants were sonicated in: 1) methanol 2) DCM, 3) DI water or 4) 0.9% saline, divided into soluble and whole particle fractions, and oxidative potential was determined using an acellular model, the dithiothreitol (DTT) assay. Comparisons between extraction technique, location, and fraction are underway. Preliminary data from a range of black carbon concentrations (0.16 to 7.35 $\mu g/m^3$) were observed across seasons with significant differences between seasons in our most urban and rural locations. Additionally, winter and spring samples indicate significant differences in DTT consumption ($nmol/min/m^3$) are observed based on the extraction solvent used. Elemental analysis using ICP-MS has also indicated variability between extraction solvent and the elements quantified, with select elements (Pb, Ni, Cs, Rb) significantly correlated with DTT consumption based on extraction solvent. With initial data, we anticipate that these results from previously collected yet untested filters will identify the importance of filter extraction method on reliability and interpretation of oxidative potential results across season.

**4.19.P-Th088 Spatially Explicit Pesticide Exposure Modeling to Inform Potential Refined, Location-Based Mitigations**

*Rochelle Bohaty,* ***Gretchen Dykes****, Sarah Hafner, Jessica L.O. Joyce, Colleen Rossmeisl, Dana Spatz and Holly Summers, U.S. Environmental Protection Agency*

Exposure modeling typically provides a general prediction for a vulnerable area, which is then extrapolated to represent a broad swath of the country. Spatially explicit modeling provides pesticide concentration predictions that are specific to a given area of the country, often at refined spatial scales (like sub-watersheds). When performed at the sub-watershed (or HUC-12) scale, spatially explicit modeling of pesticide concentrations in the aquatic environment can give greater spatial resolution to help identify areas of potential concern to listed and non-listed species. This could be especially beneficial for pesticides with a broad use footprint, or could be paired with other spatially explicit data, such as refined species ranges, to further distill the regions where mitigations may be needed. To develop refined location-based mitigation strategies, risk assessors can employ models such as EPA's Spatial Aquatic Model (SAM, currently under development) or USGS's Watershed Regression for Pesticides for Multiple Pesticides (WARP-MP) model. In addition, risk assessors can incorporate water monitoring data—even from sparse data sets—using USGS's SEAsonalWAVEQ with EXtended capabilities (SEAWAVE-QEX) model to predict peak pesticide concentrations from infrequent sampling. A case study is presented to describe approaches for HUC-12 watershed-based modeling to inform potential mitigation.

**4.20.P Poster Only: Chemistry and Exposure Assessment**

**4.20.P-We134 A Probability-based National Assessment of Contaminants in Fish from United States Rivers**

*Leanne L. Stahl[1], **John C. Healey**[1], Blaine D. Snyder[2], Harry B. McCarty[3] and Tara R. Cohen[2], (1) U.S. Environmental Protection Agency, (2) Tetra Tech, Inc., (3) General Dynamics Information Technology*

Most existing fish consumption advisories in the United States (U.S.) have been issued for mercury and polychlorinated biphenyl (PCB) contamination, but states have recently begun to issue advisories for per- and polyfluoroalkyl substances (PFAS). The U.S. Environmental Protection Agency (EPA) developed an unequal probability survey design to allow a comprehensive characterization of mercury, PCB, and PFAS contamination in fish from U.S. rivers on a national scale. During 2018-2019, fish fillet samples were collected from 290 sites selected randomly from the target population of rivers ($\geq 5^{th}$ order in size) in the conterminous U.S. This comprised a nationally representative sample and allowed extrapolation to a sampled population of 66,142 river km. The goal was to develop estimates of the national distribution of total mercury, all 209 PCB congeners, and 33 PFAS (including perfluorooctane sulfonate or PFOS) in river fish. All fillet tissue samples contained detectable levels of mercury and PCBs. PFAS were detected in 95.2% of the fillet samples. Fish tissue screening levels (SLs) applied to national contaminant probability distributions allowed an estimation of the percentage of the sampled population of river km that contained fish with fillet concentrations above a level protective of human health. Fish tissue SL exceedances for an average level of fish consumption (i.e., the general population of fish consumers) were 26.0% for mercury (applying EPA's 300 ng/g fish tissue-based water quality criterion), 17.3% for PCBs (using a derived 49 ng/g noncancer SL), 45.1% for PCBs (using a derived 12 ng/g cancer SL), 0.7% for PFOS (using a derived 46 ng/g noncancer SL). Fish tissue SL exceedances for high-frequency fish consumers (e.g., subsistence fishers) were 46.2% for PCBs (using a derived 11 ng/g noncancer SL), 73.8% for PCBs (above a 2.8 ng/g cancer SL), and 18.3% for PFOS (using a derived 11 ng/g noncancer SL).

**4.20.P-We135 Investigating the Trace Metal Leaching of Cigarette Butts in Environmental Systems**

***Anna Altmann** and Justin Clar, Elon University*

A substantial amount of research has addressed the environmental impact of nicotine from cigarettes and cigarette butts (CBs) on human and environmental health. However, very little research has involved studying the trace metals found in CBs and their impact on human and environmental health. Previous studies on the release of trace metals from CBs have not determined if CBs are a source or sink for trace metals in the environment. This research seeks to address that confusion by developing a baseline of trace metals content in CBs before subsequent environmental release studies. In Phase 1 of this project, a mass balance was completed to determine how much metal was found inside each component (paper, tobacco, filter) of an unsmoked cigarette. The cigarette components from six brands were digested with hydrochloric acid and nitric acid using EPA method 3051A before metals analysis with Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES). The metals found in the paper varied by brand, but the metal in the highest concentration in five of the six brands was iron (~300 µg/Cig). The metals measured in the tobacco samples were higher than that of the paper or filter. Specifically, the metal found in the highest concentration inside tobacco samples from every brand was magnesium. Phase II of this study compared leachate concentrations of CB samples collected around Elon University against those of artificially prepared CBs in a lab. Test fluids included DI water, SPLP, buffered solutions (pH 7, pH 10), and artificial seawater. Filtered leachates were digested using EPA method 3015A and analyzed via ICP-OES. The artificially prepared CBs and the collected CBs were found to leach similar amounts of each metal. Between 10 and 15 trace metals were detected in the CB leachates, including aluminum, copper, and zinc. Concentrations varied depending on the leaching solution used. In the future, collected CBs with more degradation will undergo leaching to determine how degradation impacts trace metal leaching. This will reveal if the leaching changes during the natural breakdown of the litter and give a more complete picture of how CBs impact the environment.

**4.20.P-We136 Phthalates and Phthalate Alternatives Analysis Using Gas Chromatography Mass Spectrometry Demonstrate with Real World Biological and Environmental Matrices**

*Kaley Adams, **Caoilinn Haggerty**, Ricky Scott and Kim Anderson, Oregon State University*

Global production of phthalates plasticizers has accelerated to six million tons per year as the demand has increased due to their advantageous properties. Phthalates are now found in a wide array of consumer products causing an interest in environmental impacts of phthalates and their public health exposures. A gas chromatography mass spectrometry (GC-MS) method was developed for over 30 phthalate and phthalate alternatives using an Agilent 8890 GC 5977B MS. The method utilizes both scan and selected ion monitoring (SIM) modes to identify and quantitate the target compounds. The method validation consisted of inter and intraday variability, demonstration of calibration with an $r^2$ value of 0.98 or better over a calibration range of 0.25 ppm to 25 ppm, limits of detection ranging from 50 ppb to 250 ppb. Method is further demonstrated with the analysis of silicone wristbands, low density polyethylene (LDPE) passive samplers, and biological tissues. We analyzed silicone wristbands worn for 48-hours and found phthalates such as diisobutyl phthalate and butyl benzyl phthalate ranged from 0.65 to 281 µg/wristband. Phthalates such as bis (2-ethylhexyl) phthalate were detected up to 30,100 µg/wristband. Alternative plasticizers such as bis (2-ethylhexyl) terephthalate were found up to 31,400 µg/wristband. Results from other matrices that will be presented include LDPE passive samplers, silicone breast explants, human plasma, smoked salmon, and bird eggs.

**4.20.P-We137 The Role of Retrospective versus Prospective Environmental Risk Assessments**

***Francis Ramacciotti**[1] and William Bullock[2], (1) GHD, (2) CSX Transportation*

Environmental risk assessment can and should play a valuable role in decision making and risk communication for numerous applications. Risk assessment is a scientific tool that has been around for many decades and is often regarded as a field that is confusing or otherwise fully comprehended by only a limited few practitioners. However, this is far from reality. Risk assessment at its core is simply an evaluation of actual or potential exposures to chemicals in a workplace or the environment (exposure) and combining that with the exposed dose (exposure assessment), plus accounting for the corresponding chemical toxicity. This is a field that is commonly practiced in industrial hygiene, as well as in environmental/contaminated site situations. Unfortunately, regardless of the application, there are instances where individuals appear to mis-communicate or misuse the exposure assessment. This appears to happen most often when practitioners move from prospective (future generic or unknown use/exposure) to retrospective (actual exposures that occurred historically) assessments, or vice versa. When this happens, the results often lead to confusion and/or distrust among the parties involved, which does not have to happen.

To eliminate this confusion, we outline some of the key differences between retrospective and prospective environmental risk assessments. We also identify where one approach is superior to (and preferred) relative to the other, as well as providing perspective on where additional interaction between these two types of assessment may be more appropriate. The evaluation makes direct reference to regulatory citations that provide the first principles basis for these types of exposure and risk assessments to serve as a quick reference guide for new and experienced practitioners. This work includes not only discussion and evaluation of specific exposure factors/assumptions, but it also provides perspective on the types of chemistry/concentration measurements typically available and how to reasonably pair chemistry and exposure to result in defensible and reliable risk characterizations that are protective and can be used to make decisions. Additionally, the recommended approach results in an easier to understand conclusions that are more digestible to those unfamiliar with the details involved in working through the risk assessment process.

**4.20.P-We138 Development of Reference Materials for the Measurement of Per- and Polyfluoroalkyl Substances in Food and Agricultural Products**

***Benjamin Place*** *and Alix E. Rodowa, National Institute of Standards and Technology (NIST)*

Recently, there have been numerous studies reporting presence of per- and polyfluoroalkyl substances (PFAS) in food and agricultural products arising from various contamination sources. PFAS have been reported in vegetables, animal feed, dairy products, and meat products. At the National Institute of Standards & Technology (NIST), there are only two food (fish tissue) reference materials with reported concentrations of PFAS, so there is a significant need for additional matrices to provide quality control for PFAS measurements in the food chain. In collaboration with state and other federal government agencies, researchers at NIST identified and acquired source material with suspected PFAS contamination including beef, pork, spinach, and corn silage. A material processing method was developed for each material to evaluate the effect of processing on sample handling and targeted PFAS measurements. Each processed material was screened for targeted PFAS, and measurable quantities of individual compounds were detected in all samples, notably PFOS was detected in all samples. The materials varied in total concentration and number of PFAS, which may be related to the material matrix and source of PFAS contamination. In addition, the materials were tentatively screened for compounds that could interfere with accurate PFAS measurements. All materials are intended to become publicly available reference materials for commercial and research laboratories to evaluate targeted PFAS methods. The estimated timeline for the bulk processing, packaging, measurement, and release of the material will be presented.

**4.20.P-We139 Saturate, Aromatic, Resin and Asphaltene (SARA) Analysis of Floating Oil Exposed to UV Light**

***Emily C. Pisarski*** *and Ed Wirth, National Oceanic and Atmospheric Administration (NOAA)*

The photo-oxidation of oil is becoming more recognized as an important weathering process after oil is spilled in the environment, occurring in the hours to days post-oil spill rather than during the weeks to months as previously thought. Understanding how oil weathers under solar irradiation can improve models that forecast the fate and transport of oil spills and also better inform oil spill responders on the types of cleanup and mitigation techniques to use. This study builds off previous work which examined the chemical and physical changes that occur in floating oil (Louisiana Sweet Crude; LSC) exposed to ultraviolet light (UV-A) at different temperatures. Initial results indicated that oil exposed to UV light formed tarball-like features, regardless of temperature, whereas non-UV exposed oil generally remained sheen-like throughout the duration of the exposure. However, from a chemical standpoint, only minor changes, related to polycyclic aromatic hydrocarbons, were detected. This next phase of research explores how the saturate, aromatic, resin, and asphaltene fractions (SARA analysis) change as oil is exposed to UV light and different temperatures (10, 21 and 30°C) over a ten day period. Physical changes in the oil will be documented through the use of photography. Preliminary results with LSC at 21°C indicated that there was greater asphaltene content in the UV treated oil, which corresponded to tarballs being formed. In the no-UV treated oil, asphaltene content did increase over the ten day period, but it was less than the UV treated oil (10.3±3.2% versus 15.4±2.0%, no UV and UV treated, respectively). Results from this study will be used to improve models that forecast the fate of floating oil in the environment and inform future directions for additional research.

**4.20.P-We140 Development of field test to determine Zinc contamination**

***Eric Lee*** *and Cidney Cardenas, M-I SWACO, a Schlumberger company*

The drilling fluids toxicity test (EPA 1619) has served as a compliance tool to determine the end of pipe toxicity of water-based drilling fluids. The engineering challenge of using the test is to predict compliance with the discharge limit before discharging. In most cases, a combination of drilling fluid product knowledge and onsite testing for oil and chlorides are used to predict compliance with the regulatory limit prior to discharge. However, this commonly used engineering approach does not manage specific contaminants that may be introduced to the fluids during the drilling process. For example, zinc bromide ($ZnBr_2$) is a completion fluid brine that is an uncommon contaminant in drilling fluids. Zinc is a Clean Water Act priority pollutant and Zinc bromide can cause a compliance failure at low concentrations. Some laboratory methods to test for zinc include titrations, which can be infeasible in field conditions. There are, however, commercially available test strips kits that can quickly test for soluble zinc.

The main objective of this study is to develop a field screening tool that can detect total soluble zinc in water-based drilling fluids to stay in compliance in accordance to the NPDES permit. Preliminary results from the test strip kits are provided with corresponding analytical laboratory analysis to show the confidence in the strips and techniques.

**4.20.P-We141 What is "Background" for Wetland Habitats in Urban Areas? The Role of Equilibrium Partitioning**

***Brian S. Yates*[1]** *and W.J. (Jim) Rogers[2], (1) Yates Environmental Sciences Inc., (2) West Texas A&M University*

Organic and inorganic contaminants are ubiquitous in freshwater and tidal habitats surrounded by urban development. In regulatory clean-up programs, background data are frequently published for soil to support no further action (NFA) determinations. However, such data are not always appropriate when comparing to sediment. Sediment background data are sparse and often don’t include accompanying geochemical data such as acid-volatile sulfide (AVS), organic carbon and grain size. In the northeastern United States, it’s not uncommon to find diffuse metals and polyaromatic (PAH) concentrations in sediment near urban areas several times higher than background soil levels, regardless of whether a point-source discharge has occurred. Review of a large background dataset indicates that equilibrium partitioning explains a great deal of the variation in bulk sediment concentrations. Freshwater and tidal marshes frequently contain upwards of 20 to 50% total organic carbon (TOC), whereas background soils often contain less than 3%. Similarly, AVS is often nonexistent in upland mineral soils, but ubiquitous in marshes, quiscient streams and other depositional systems. Such catchments often act as “sinks” for diffuse sources of anthropogenic pollution. Simultaneously, the same elements that attract contaminants (fine-grained sediment, AVS and TOC) reduce their bioavailability. To summarize, it is paramount that both practitioners and regulators consider particle grain size, AVS and TOC when comparing sediment data to background. Ecological clean-up levels developed for sediment using background soil data are inappropriate if they do not adequately capture the range of variability that is associated with AVS, organic carbon and grain size.

**4.20.P-We142 How Much Persistent Organic Pollutants Have Ever Been Produced and Released into the Global Environment?**

***Li Li*[1]***, Chengkang Chen[2], Dingsheng Li[1] and Yi-Fan Li[3], (1) University of Nevada, Reno, (2) Peking University, China, (3) Harbin Institute of Technology, China*

Information on the global production and environmental releases of persistent organic pollutants (POPs) is of critical importance for regulating and eliminating these persistent, bioaccumulative, toxic, and globally ubiquitous chemical substances. In this work, we conduct an extensive literature review to collect and curate information on the historical global production and multimedia environmental releases of 25 intentionally produced POPs subject to elimination and restriction by the Stockholm Convention. Our assembled data indicate that as of 2020, a cumulative total of 31,306 kilotonnes (kt) of the 25 POPs had been synthesized and commercialized worldwide, and a cumulative total of 20,348 kt had been released into the global environment. Short-chain chlorinated paraffins are the most produced POP in history, with a global cumulative tonnage amounting to 8,795 kt by 2020, whereas α-hexachlorocyclohexane has the largest historical cumulative environmental releases of 6,567 kt by 2020 among these 25 POPs. The 1970s witnessed the peak in the annual global production of these POPs. The U.S. and Europe used to be the hotspot of environmental releases of these 25 POPs, notably in the 1960s and 1970s, whereas the environmental releases of POPs occurred primarily in China in the most recent decade. We also quantify the global total environmental impacts associated with the production of these chemicals by calculating "equivalent" tonnages of these 25 POPs, based on their toxicity (the human reference dose) relative to that of dichlorodiphenyltrichloroethane. The results show that although the global cumulative production of perfluorooctanesulfonic acid and related substances was relatively limited, their equivalent tonnage was remarkable (ranked second among the 25 POPs) and even close to that of the heavily produced polychlorinated biphenyls.

**4.20.P-We144 Exposure Assessment in the Subarctic: Lead in the Sahtú, Northwest Territories, Canada**

***Calin Lazarescu**[1], Mylene Ratelle[1], Larisse Melo[2,3], Yvonne Lamers[2,3], Kelly Skinner[1] and Brian Laird[1], (1) University of Waterloo, Canada, (2) University of British Columbia, Canada, (3)BC Children's Hospital Research Institute, Canada*

Lead (Pb) is an element that is toxic to humans in high concentrations. Pb is still widespread in environmental media including soil, air, and water, due to anthropogenic releases into the environment, as well as its use in industry and hobbies. The Sahtú is a subarctic region located in the Northwest Territories, Canada, and over three-quarters of the population is Indigenous, with most of the Indigenous population being Dene.

In the Sahtú, average blood Pb biomarker levels were found to be higher than those in the general Canadian population, as assessed through the Canadian Health Measures Survey, and over 20% of the population sampled had blood Pb levels greater than 5ug/dL; it is not yet clear why levels are high.

This project aims to collect and compile information on possible Pb determinants related to exposure pathways. Routes of exposure are being assessed through various methods including statistical analysis of biomarker and nutrient levels, the development and feedback gathering of an individual questionnaire, and literature review.

Possible routes of exposure resulting in elevated Pb levels include diet (through the consumption of traditional food containing Pb including birds who are exposed to Pb via long-range transport, and low nutrient levels resulting in elevated Pb absorption), hunting practices (such as the use of Pb ammunition, eating the meat around wound channels, and inhalation of expended shot), and other exposure sources (such as the use of Pb in household products and use in hobbies).

Statistical analysis indicates nutrient biomarker levels (Zinc, Vitamin D, Iron) have significant correlations with Pb levels and are contrary to the established physiochemical interactions between Pb, Zn, Fe, and Vitamin D since nutrient and Pb concentrations increase together. A regression model was created with age, sex, smoking status, and zinc concentrations as explanatory variables for predicting Pb levels. Further, community outreach and survey refinement indicate Pb ammunition may still be used in hunting and that household Pb exposure may be prevalent.

The research generated will directly address community concerns, who have expressed concern in identifying why Pb levels are high and methods in reducing Pb concentrations in the future. Previous work on biomarkers in the region has not been completed in decades, and the results of this work will be used for the future development of a Pb monitoring program in the region.

**4.20.P-We145 Characterizing and Quantifying Chemical Ingredient Use in Consumer Products Between Two Separate Databases and Implications for Environmental and Human Health Exposure**

***Todd Gouin**, TG Environmental Research, United Kingdom*

Assessing chemical exposure in home and personal care products (HPCPs) represents an important data need. Key challenges to the assessment are related to limited knowledge quantifying and characterizing the weight-fraction inclusion level and functionality of chemicals in HPCPs. Publicly available tools have been developed to address these challenges, such as the Chemical and Products database (CPDat). This study aims to evaluate the relative performance of CPDat by comparing estimates of weight-fraction inclusion level and functionality to other relevant data sources. Specifically, estimates obtained from CPDat are evaluated and compared with estimates obtained from marketing analytic data, using Euromonitor Passport for 31 commonly used chemicals found in HPCPs. The results obtained from this exercise suggest relatively good agreement between each of the methods for 10 chemicals. For the remaining group of chemicals, notable differences are observed, which are attributed to differences in how the underlying data are obtained for each of the methods. With an emphasis on obtaining data based on mining data-sheets for individual products, application of CPDat is suggested to be useful for higher tiers of assessment, with data obtained from marketing analytics providing valuable input to exposure-based screening models. Insight gained from this study can be used to help guide the appropriate use of data obtained from different sources within a tiered exposure assessment.

**4.20.P-We146 Addressing the Importance of Microplastic Particles as Vectors for Long-Range Transport of Chemical Contaminants: Perspective in Relation to Prioritizing Research and Regulatory Actions**

***Todd Gouin**, TG Environmental Research, United Kingdom*

Over the last several years there has been increasing concern regarding the environmental fate and potential global transport of plastic debris, particularly in the form of microplastic particles (MPs). The global transport of MPs has also triggered concerns regarding the potential role that its mobility may represent towards influencing the long range environmental transport (LRET) of particle-bound chemicals, particularly the large number of chemicals known to be added to plastic. This perspective considers the various lines-of-evidence that might be used towards understanding the LRET of persistent organic pollutants (POPs). For instance, it has been proposed that the LRET of POPs is facilitated by global fractionation processes that facilitate the mobility of chemicals from source regions towards remote locations, such as the polar regions, where they have the potential to accumulate. These processes are influenced by the physicochemical properties of POPs and can result in various transport mechanisms influencing environmental fate and transport. Here I suggest that there are similarities that can be drawn, whereby knowledge of how differences in the physicochemical properties of MPs relative to different emission scenarios, can influence the relative importance of sequestration processes that may result in global fractionation of MPs. Several challenges are identified throughout the perspective, with an urgent need towards the development and application of standard sampling and analytical methods being identified as critical for enabling datasets to be reliably compared for use in better understanding potential source-receptor relationships, as well as advancing the characterization and quantification of various environmental fate processes. In many instances, it is suggested that advances in our understanding can be facilitated based on knowledge obtained in other areas of research, such as in relation to studies developing tools to evaluate the mobility of particulate organic matter in aqueous environments or from studies investigating the fate and mobility of atmospheric particulates. Recognizing that not all MPs are equal, with respect to environmental fate and toxicological effects, knowledge regarding which types of MPs are likely to be subject to LRET can only strengthen our ability to evaluate their role as vectors of transport for plastic associated chemicals and the associated risks that their LRET may represent.

**4.20.P-We147 Identifying PCB sources and reduction needed to achieve fish concentration target using passive sampling**

***Nathalie Lombard**[1], Leigh Anne Weitzenfeld[2] and Upal Ghosh[1], (1) University of Maryland, Baltimore County, (2) Public Work Department*

Like many waterbodies in the United States, the Roanoke River is still impaired by polychlorinated biphenyls (PCBs). PCB load reduction from point and non-point sources is needed to restore water quality standards protective for the fishing designated use of the river.

To identify the main sources leading to elevated PCB concentration in fish, we implemented a monitoring program using low density polyethylene passive sampler. PCB concentration was measured in water column, sediment porewater and air. Fluxes between sediment and water and between water and air were calculated, as well as dissolved PCB loads from tributaries using USGS discharges data. Using the fluxes and loads, a PCB mass balance was developed for the Roanoke River to identify sections where majority of the PCBs are entering the water column. This approach allowed identification of the two major tributaries (Peters Creek and Tinker Creek) as well as a minor tributary (Ore Branch) as the main contributors to PCB loads into the river while bed sediment and atmosphere had minimal impact.

PCB reduction in water column needed to achieve fish concentration target in Roanoke River was further estimated using a thermodynamic equilibrium model. The model was first validated by comparing bioaccumulation predicted using water column passive sampling data against measured concentrations in a variety of fish collected the same year. Total PCB concentration in fish was predicted within a factor of 2, though slight under prediction was observed for higher trophic level fish. Based on the thermodynamic equilibrium model, fish within Roanoke River and Tinker Creek were close or below the Total Maximum Daily Load (TMDL) fish concentration target, suggesting that no PCB reduction in water column is needed. However, 45 to 85% PCB reduction would be needed in the water column of respectively Peters Creek and Ore Branch.

**4.20.P-We148 A Multiple Linear Regression Approach for the Estimation of Carboxylic Acid Ester and Lactone Alkaline Hydrolysis Rate Constants**

***Jovian Lazare***, *Caroline Tebes-Stevens and Eric Weber, U.S. Environmental Protection Agency*

Predictive models are important to regulatory organizations such as the U.S. Environmental Protection Agency (EPA) for addressing data gaps in chemical risk and exposure assessment. Quantitative Structure Activity Relationships (QSARs) can be used to estimate physicochemical properties and transformation rates when experimental values are lacking. A multiple linear regression (MLR) approach is used to develop robust QSARs for predicting base-catalyzed hydrolysis rate constants of carboxylic acid esters and lactones. The approach is intuitive and easy to interpret. Models are being developed primarily based on underlying concepts from linear free energy relationships (LFER) and the use of electronic parameters for reactivity. These parameters include protonation ($pk_A$), charge (and electronegativity), Hückel analysis (charge density), and steric (both topological and geometrical) parameters. Various combinations of independent descriptors are being explored collectively. The developed models have shown significantly improved performance compared to some popular hydrolysis models (HYDROWIN and SPARC). The models will be implemented in EPA's Chemical Transformation Simulator (CTS) platform, and a similar approach will be used to develop QSAR models for predicting transformation rates of chemicals with other hydrolyzable functional groups.

**4.20.P-We149 Measuring On and Off-Duty Exposures of Structural Firefighters with Silicone Passive Samplers**

***Emily Bonner***[1], *Carolyn Poutasse*[1], *Walker Poston*[2], *Christopher Haddock*[2], *Sara Jahnke*[2], *Lane Tidwell*[1], *Peter Hoffman*[1] *and Kim Anderson*[1], *(1) Oregon State University, (2) National Development and Research Institutes, Inc.*

Firefighters serve a critical role in protecting communities, yet suffer from occupational exposures to a range of chemicals while on the job. Chemicals in furniture, building materials, and home goods can be volatilized during a building fire; pyrogenic chemicals can also be produced. Such occupational exposures have been associated adverse health outcomes such as cardiovascular disease and various cancers. We recruited 57 firefighter participants from two fire stations in the Kansas City Metropolitan area to wear silicone dog-tags around their necks as passive samplers. We analyzed the tags for chemicals with suspected relevance to firefighters using gas chromatography, mass spectrometry. Analytes included 43 polychlorinated biphenyls (PCBs) and 21 other volatile organic compounds (VOCs). Separate on- and off-duty tags were each worn for a total of 30 days (24 hours a day) when on-duty or off-duty to compare individual chemical exposures. We also compared exposures between stations because on average, one of the stations received fewer than two calls per month (low call volume), and the other received more than 12 calls per month (high call volume). It was hypothesized that chemical concentrations in paired on-duty versus off-duty tags would be higher, that the concentrations in high versus low call volume station tags would be higher, and that dog-tag concentrations would be influenced by the questionnaire variables (firefighter rank, number of fire attacks, etc.). On- versus off-duty exposures to PCBs are highly individual, with no significant difference in concentration in relation to duty-status. Only 28% of firefighters had detectable concentrations of PCBs in either sample; 40% (n=30) of high call volume station firefighters and 15% (n=27) of low call volume station firefighters had detectable PCB exposures, supporting the hypothesis that the high call volume station firefighters had greater exposures. Additionally, 12 different PCB congeners were detected, with PCB 153 being the most frequently detected. 17 VOCs have been detected in samples analyzed so far (n=20). The ease of sample collection and data produced in this study demonstrates the value of using silicone passive samplers as a tool in assessing firefighters' chemical exposures and occupational risk.

**4.20.P-We150 Polycyclic Aromatic Hydrocarbon Concentrations in Plasma Samples are Associated with Cardiovascular Risk Biomarkers in a Near-Roadway Study Human Population**

***Caitlin Johnson***[1]*, Katherine Manz*[2]*, Mei Chung*[3]*, Doug Brugge*[4] *and Kurt Pennell*[2]*, (1) Geosyntec Consultants, Inc., (2) Brown University, (3) Tufts University, (4) University of Connecticut*

Polycyclic aromatic hydrocarbons (PAHs) are air pollutants associated with multiple adverse health effects in humans. PAHs exposure in humans is typically determined by measuring hydroxylated PAH metabolites in urine, but little is known about plasma concentrations of un-metabolized PAHs. The goal of this study was to quantify 15 PAHs in the plasma from a near-highway study population and assess relationships between plasma PAH concentrations and cardiovascular risk biomarkers.  Plasma samples from 58 non-smokers were analyzed using high-resolution gas chromatography mass spectrometry. Anthracene, phenanthrene, fluoranthene, pyrene, and benzo[*ghi*]perylene were detected in > 70% of plasma samples and their joint relationship with each of four cardiovascular risk biomarkers (interleukin-6 (IL-6), c-reactive protein (hsCRP), tumor necrosis factor receptor II (TNF RII), and fibrinogen) was investigated using weighted quantile sum regression and Bayesian kernel machine regression. Models of IL-6 indicated positive ($p < 0.001$) nonlinear relationships with benzo[*ghi*]perylene, fluoranthene, and pyrene. For hsCRP, positive nonlinear associations ($p < 0.05$) were found with anthracene, phenanthrene, and benzo[*ghi*]perylene; however, fibrinogen and TNF RII were not significantly ($p > 0.05$) associated with any plasma PAH concentrations. Results of this study suggest PAH levels in human plasma are associated with biomarkers of cardiovascular risk and support further evaluation of relationships between PAHs in plasma and adverse health effects.

**4.20.P-We151 Wastewater, Fish Tissue and Biosolids - An Analytical Evaluation of EPA Draft Method 1633**

*Megumi Shimizu*[1]*, Sam Lodge*[2]*, Philip Bassignani*[3] *and* ***Matthew Noestheden***[1]*, (1) SCIEX, (2) Phenomenex, Inc., (3) Alpha Analytical*

EPA draft method 1633 covers the extraction and analysis of 40 per- and polyfluoroalkyl substances (PFAS) in a variety of environmentally relevant matrices.  As a method not yet promulgated, the test method procedure and method performance requirements are not part of the clean water act, but it is currently be offered by many commercial laboratories. Here we evaluate the performance of the draft method procedure in wastewater, fish tissue and biosolids.  Full initial demonstration of capability (IDC) data are provided including method detection limits and precision in each matrix evaluated.  The full test method was applied to the analysis of real world samples and the resulting data are presented.

**4.20.P-We152 Optimized Sample preparation and Sensitive Analysis for Per and Polyfluorinated Alkyl Substances (PFAS) in Whole Blood**

***Emily Parry**, Jennifer Hitchcock and Limian Zhao, Agilent Technologies, Inc.*

Per and polyfluoroalkyl substances (PFAS) are a general class of anthropogenic compounds that contain high degree of fluorination. The richness in carbon-fluorine bonds makes PFAS valuable in a variety of industrial manufacturing processes, however enhances their resistance to degradation. These properties have led to their ubiquitous presence in the environment. Human exposure and PFAS body burden have been linked with a variety of negative health effects and research is ongoing. Epidemiological studies rely on the quality of the analytical data which has been complicated by the diversity of molecular structures in the PFAS class. A variety of methodologies for PFAS sample preparation in biological matrices have been reported such as direct protein precipitation (PPT), solid phase extraction (SPE), PPT followed with Enhanced Matrix Removal Lipid (EMR-Lipid) cleanup etc. The current study presents an optimized sample preparation and LCMS detection targeting 40 PFAS compounds in whole blood on LC/MS/MS. The whole blood sample contains high amounts of proteins and the sample volume and crashing solvent for PPT extraction was optimized. Direct PPT was compared with PPT followed by EMR-Lipid cleanup. EMR-Lipid cleanup demonstrated the significantly reduced matrix effects, while providing acceptable targets recoveries. LCMS conditions were optimized to achieve low detection levels which ranged from 0.03 – 0.67 ng/mL. Focus of this work was targeted analysis, however, the sample preparation procedure would serve well for non-targeted analysis as EMR-Lipid cleanup doesn't retain any PFAS functional group (acids, ethers, etc.) and allows for the identification of new biologically relevant PFAS compounds.

**4.20.P-We153 Determination of heavy metals in the cargo port of the bay of Cartagena, Colombia**

***Fredys Sanchez**[1], Jessica Mercado-Páez[2], Gisela Mayo-Mancebo[1], Natalia Burgos-Uribe[1] and Farith Damian Gonzalez[2], (1) Centro de Investigaciones Oceanográfias e Hidrográficas del Caribe (CIOH), Colombia, (2) University of Cartagena, Colombia*

Heavy metal contamination in marine sediments is an issue that affects and generates concern in all countries with maritime territory due to the use of different activities such as industry and tourism. The bay of Cartagena, due to its geographical location, has been used for many years for cargo transportation. Reason for which the content of Lead (Pb), Chromium (Cr), Cadmium (Cd), Cobalt (Co), Copper (Cu), Nickel (Ni) and Zinc (Zn) was determined in sediments taken near a cargo port in the Mamonal area in the department of Bolivar. The samples were taken with the help of a dredger for the collection of the sediment and the analyzes were carried out in an atomic absorption equipment by the flame method. Obtaining the following average values for each of the studied metals; Lead (8.77 ppm), Chromium (40.41 ppm), Cadmium (0.08 ppm), Cobalt (5.37 ppm), Copper (90.91), Nickel (23.43) and Zinc (127.02). The presence of these metals in the environment is due to both natural and anthropogenic sources: industrial and domestic waste, agriculture, leaching and atmospheric deposition, naval and coastal human activities. These metals are considered environmental contaminants due to their capacity for bioaccumulation, toxicity and persistence, which mainly and directly affects aquatic systems. Therefore, one way to know the degree of contamination of a body of water is to evaluate the quality of the sediments, since they have a high capacity to store heavy metals.

**4.20.P-We154 Batch Equilibrium Experiments Indicate Weak Temperature Dependence of Soil/Sediment-Water Sorption for Cyclic Volatile Methyl Siloxanes**

***Gary Kozerski**, Jeremy Durham and Jaeshin Kim, The Dow Chemical Company*

The organic carbon normalized partition coefficient, $K_{OC}$, describes the equilibrium distribution of a chemical between water and particulate organic matter; it is a key parameter in many chemical fate and transport models. In recent years, numerous studies have been published pertaining to environmentally relevant physicochemical properties of cyclic and linear volatile methyl siloxanes (VMS), including $K_{OC}$. Studies of the sorption behavior of several VMS substances between water and soil or sediment have been reported, with $K_{OC}$ values determined either directly using the batch equilibrium method, or indirectly from measurements of volatilization rates from particle-water slurries. Previously, the temperature dependencies of $K_{OC}$ values for the cyclic VMS compounds octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5) have been investigated only by the indirect purge-and-trap method.

The present study examined the $K_{OC}$ temperature dependencies of D4 and D5 by the batch equilibrium method. Despite unknown variations arising from use of sediments of differing origin, empirical $K_{OC}$ values reported for 25 °C by the different methods agreed to within approximately 0.5 and 0.7 log units for D4 and D5, respectively. However, at lower temperature of 4-5 °C, these differences increased significantly, with batch equilibrium $K_{OC}$ values lower by 1.8 log units for D4 and 1.3 log units for D5. Whereas the $K_{OC}$ values by the purge-and-trap method increased with decreasing temperature, giving sorption enthalpies ($\Delta H_{OC}$) of -79 kJ/mol for D4 and -48 kJ/mol for D5, the batch equilibrium $K_{OC}$ values showed modest decreases at lower temperatures with corresponding $\Delta H_{OC}$ values of 0 (i.e., not statistically significant) to +13 kJ/mol. These findings could have significant implications for the predicted fate of D4 and D5 in colder environments particularly.

Ongoing work is exploring how experimental differences between the studies, such as sorbent-water ratios or preparation and spiking of sorbents, might contribute to the observed discrepancies in $K_{OC}$ values. Additionally, the dynamic method is being assessed from a theoretical perspective to identify potential bias arising from assumptions about the behavior of VMS materials in the purge-and-trap system. It is expected that the current study will be an important step toward resolving these apparent discrepancies, and eventually defining consensus values of these important parameters for the VMS substances.

**4.20.P-We155 Major uncertainties in predictive assessment on fate and transport of volatile methylsiloxanes**

***Jaeshin Kim*[1] *and Michael Whelan*[2]*, (1) The Dow Chemical Company, (2 )University of Leicester, United Kingdom***

Multimedia fate and transport models (MFTMs) have long been employed to predict chemical behavior and exposure in the environment, along with associated ecotoxicological risk assessment, and to estimate persistence, long-range transport, and bioaccumulation potential. These models require a set of inputs such as the inherent properties of the chemicals under investigation and the characteristics of the receiving environmental systems. It is critical to make accurate estimations of these parameters to ensure that model predictions are reliable. Accordingly, we evaluated generic aspects and applications of MFTMs to better understand the behavior of volatile methylsiloxanes (VMS) in the environment.

The physico-chemical properties of VMS compounds differ markedly from those of most carbon-based organic compounds, which can have a major effect on their behavior. Thus, the properties should be properly incorporated into MFTMs. Good agreements between modeled and measured concentrations in air, sediment, and biota indicate that our general understanding of the environmental fate of VMS is reasonable: VMS compounds are “fliers” that principally partition to the atmosphere but have low redeposition potential. They are degraded in air by reacting with OH radicals with half-lives of 3–10 days, which means that they have high characteristic travel distances but low overall persistence and low target-oriented long-range transport potential. Since they are also released to water and soil via the wastewater and biosolids-to-land pathways, VMS have been detected in these compartments, where exposure can be limited by hydrolysis, volatilization, and partitioning to sediments. In soil, concentrations are reduced by volatilization and clay-catalyzed hydrolysis. In aquatic food webs, metabolism in biota tends to drive trophic dilution resulting in trophic magnification factors which are often (but not always) <1.

Three key areas where model uncertainties still need to be addressed include: (i) the strength and direction of the temperature dependence for $K_{OC}$ which will improve confidence in model predictions of partitioning, particularly in cooler regions; (ii) the fate of atmospheric reaction products including silanols which can be deposited to surface compartments and (iii) global variations in the magnitude of emissions to wastewater which will vary with socioeconomic status and regulatory restrictions on chemical use.

**4.20.P-We156 Read-Across: A Promising Tool for Predicting Removal of Chemicals of Emerging Concern from Wastewater**

***Patricia Clyde****, Jessie M. Kneeland and Anya Sita Chinniah, Gradient*

Poor removal of many chemicals of emerging concern (CECs) in conventional wastewater treatment plants is a primary contributor of CECs to the environment *via* wastewater effluent discharge. One challenge in the research of CEC removal from wastewater is the vast number and diversity of compounds classified as CECs, each of which requires experimental testing to elucidate both overall removal efficiency and specific behavior, such as susceptibility to bio- or abiotic degradation or partitioning to sludge. Read-across analysis, a tool commonly used for toxicological risk assessment, involves the identification of relevant similarities between two or more chemicals and the reliance on data from previously studied compounds (referred to as "analogs" or "surrogates") to predict the behavior of related (typically similarly structured) target compounds. We propose that read-across may be a valuable tool for predicting the fate of CECs in wastewater treatment systems, specifically through grouping CECs based on structural similarity and physicochemical properties, and examining results from previously researched surrogates. The case study presented herein involves predicting the removal pattern(s) of multiple beta-blocker pharmaceuticals using various read-across methods. The read-across results are then compared to experimental observations reported in the literature. The results of this study allow us to examine the capabilities and limitations of read-across as a tool for efficiently applying available research results to improve our understanding of CEC removal from wastewater. The novel application of read-across to this field could largely reduce the amount of experimental research needed to predict and understand the removal behaviors of individual compounds in a vast and chemically diverse group of compounds.

**4.20.P-We157 Movement of PAHs and Alkylated PAHs Between Air and Soil Before, During, and After Wildfires**

***Kelly O'Malley****, Christine Ghetu, Diana Rohlman, Brian Smith, Ricky Scott, Kaley Adams, Peter Hoffman and Kim Anderson, Oregon State University*

In recent years, the number of large wildfires over 1000 acres in the Western United States have been increasing. Wildfire smoke is a complex mixture that is influenced by many factors including fuel type and temperature. Understanding wildfire smoke composition and movement from wildfires events is needed to inform risk assessment about wildfire events. Recent work identified vapor-phase polycyclic aromatic hydrocarbons (PAHs) and alkylated PAHS that significantly increased during wildfires and when the Air Quality Index (AQI) increased, yet little is known about PAHs movement surrounding wildfire events. Diffusive flux measures the direction and magnitude of a chemical that moves during a time interval, which can be applied to explore the movement of PAHs and alkylated PAHs between soil and air. To measure diffusive flux in wildfire smoke before, during, and after wildfires, paired soil-poreair and air low density polyethylene (LDPE) passive samplers were deployed in California, Oregon, and Washington and samples were analyzed for 42 PAHs and 22 alkyl-PAHs using a gas chromatography mass spectrometry (GC-MS/MS) method. We hypothesized that PAHs and alkyl-PAHs will deposit into the soil during high AQI wildfires and that they will volatilize after wildfires. We found that 2 ringed PAHs were found in both the soil and air before the wildfire. During wildfires, 2 ringed PAHs were mostly diffusing into the air while 4 ringed PAHs were deposited into the soil in low AQI conditions. When the AQI was above 200, 3-7 ringed PAHs were deposited in the soil during wildfires. Interesting after wildfires, 3-7 ringed PAHs volatilized from soils. This study examines both the direction and magnitude of PAHs and alkyl-PAHs movement during all stages of a wildfire events.

**4.20.P-We158 Examination of Potential Environmental Justice Issues of Community Exposure to Water Pollutants Across Counties in the United States**

*Patrick Hurbain and **Dingsheng Li**, University of Nevada, Reno*
Environmental justice (EJ) issues have a long history in the U.S. with disadvantaged communities often suffer from higher environmental pollutant exposures. Water bodies in have not been extensively studied for potential EJ issues across the U.S. Such issues may be examined with the U.S. EPA's National Lakes Assessment (NLA) and National River Assessment (NRA), which are statistical surveys of the conditions of U.S. lakes, ponds, reservoirs, and rivers. The latest available data (2017 NLA and 2018 NRA) were analyzed using generalized linear models bound to county FIPS codes for the potential of EJ issues with water bodies across U.S. counties. Social demographic status (SDS) factors including race makeup, median income, and education attainment for each county were matched using the 2010 US census data. Lead, mercury, chromium, vanadium, dioxin-like polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAH) concentrations were in water bodies were matched to the above mentioned SDS factors. Concentrations of congeners of PCBs and PAHs were transformed using respective Toxicity Equivalence Factors before aggregated into one value considering different congeners' toxicity and concentrations. A statistically elevated increase of chromium concentrations in increasing fractions non-white individuals relative to white individuals among analyzed counties was observed using univariate regression ($p=0.0162$). The same association with increasing concentrations black to white households ratio among analyzed counties was also observed using multivariate regressions ($p=0.0446$). However, no other statistically significant associations between other pollutant concentrations and SDS factors were observed. Results indicate potential EJ issues with respect to chromium exposure but overall, no evidence of significant EJ issues for other pollutants among analyzed counties' lakes and rivers.

## 4.20.V Poster Only: Chemistry and Exposure Assessment

### 4.20.V-01 Biological and environmental factors affecting radium 226 levels in soft tissue and shell of American oysters (crassostrea virginica).
***Samuel Banville** and Olivier Clarisse Clarisse, Universite de Moncton, Canada*
American oysters (*crassostrea virginica*) are currently used as biosentinel for metals such as mercury, zinc, and arsenic[1,2] and have been proposed lately as a bioindicator for radium (Ra)[3], a radioactive element. As an alkaline earth element, Ra follows calcium physiological pathways in oyster soft tissue and shell. However, Ra uptake by oyster may vary depending on environmental and biological factors. For instance, oyster's size and age may affect Ra bioaccumulation, level and distribution between soft tissue and shell. To investigate these potential effects, 1700 oysters were collected from 5 oyster farms located in New Brunswick, Canada. For each farm, around 40 four years old, 60 three years old, 80 two years old and 160 one year old oysters were shuck and pooled to obtain for each age group 6 samples with at least a 3 g dry mass of soft tissue. Corresponding shells were measured, grounded, homogenized and pooled to procure 6 other samples per age group. Acid mineralisation ($HNO_3$:HCl, 2:1) of the whole soft tissue sample and a 1.6 g shell subsample was done and the digestate were preconcentrated on a cationic exchange resin and a strontium specific resin. Ra level and distribution within each age group and oyster size will be discussed to investigate a potential relation with these biological factors. A systemic comparison of Ra results with alkaline elements (Na, Li, K, Rb), alkaline earth elements (Mg, Ca, Sr, Ba), essential elements (Zn, Fe, Zn, etc.) and non-essential elements (Cd, Cr, U, etc.) will be discussed as well for a better understanding of Ra bioaccumulation by a living organism.

### 4.20.V-02 Potentially Sacrificing Human and Environmental Health for Food Security
***Lohan Bredenhann**, Suranie Horn and Rialet Pieters, North-West University, South Africa*
South Africa is the most prolific pesticide consumer in Sub-Saharan Africa, with glyphosate-based herbicides (GBHs) being the most widely used. In eight years, the use of these herbicides more than doubled, from 3721 tonnes in 2009 to 7977 tonnes in 2017. However, because of the perceived risk to non-target biota, many governments have banned the use of GBHs. Furthermore, the South African Cancer Association agrees with the WHO that glyphosate is a potential human carcinogen. However, there is little information available on the amounts of glyphosate and its primary metabolite, aminomethylphosphonic acid (AMPA), in the South African

environment. In addition, there are no environmental regulations for these chemicals in the aquatic environment. We looked at why GBH use is so high: the widespread use of herbicide-tolerant crops and crop-rotation practices, as well as their unintended consequences, such as contributing to an already polluted river system and promoting weed resistance. This research highlights for the first time the widespread use of GBHs in South Africa, where they were sprayed on four crops in 2017–maize, soybean, wheat/barley, and sunflower–and we recommend regular monitoring for GLY and AMPA in the South African environment, despite the fact that there appears to be no evidence of risk to local human and animal health.

**4.20.V-03 Occurrence of Organic UV Absorbents in the Deepwater Redfish (Sebastes mentella) from the St. Lawrence Estuary and Gulf**

***Fella Moualek**[1], Zhe Lu[1], Dominic Belanger[2], Dominic E. Poton[2], Marc Amyot[2], Dominique Robert[1], Geneviève Parent[3] and Mathieu Babin[1], (1) Institut des Sciences de la Mer de Rimouski, Canada, (2) University of Montreal, Canada, (3) Fisheries and Oceans Canada, Canada*

Organic UV absorbents are used to protect human skin or materials from UV radiation-induced degradation in a variety of products such as sunscreens, personal care products, and plastics. Some of these contaminants have the potential to be persistent, bioaccumulative, and toxic in the environment. The Stockholm Convention on Persistent Organic Pollutants, for example, has added 2-(2H-benzotriazol-2-yl)-4,6-di-*tert*-pentylphenol (UV328) to Annex D, and a lack of environmental exposure data may impede understanding of the fate and risk assessment of these contaminants. The deep-sea (>200m) is thought to be the final reservoir for persistent contaminants in the ocean. However, the occurrence and fate of UV absorbents in the deep-sea are unknown. To this end, this study aims to use Deepwater redfish (*Sebastes mentella*) from the St. Lawrence estuary and gulf (SLEG) in Canada to investigate the accumulation potential of these contaminants in the fish from the deep-sea environment. The redfish ($n$ = 120) were collected in 2019 through the annual marine life survey conducted by Fisheries and Oceans Canada. The muscle samples were lyophilized and homogenized. Acetonitrile was used to extract the samples, followed by the cleanup of extracts using QuEChERS method. The samples were analyzed by GC-MS. Preliminary results of seven samples detected the presence of UV328 in the muscle of redfish, indicating that these contaminants could transport to the deep-sea and accumulate in the deep-sea food web. The remaining samples are currently being analyzed for UV absorbents. This poster will present preliminary findings on UV absorbents in Deepwater redfish from the SLEG. These results will provide a baseline for future monitoring of these contaminants in the deep-sea.

**4.20.V-04 Concentration of HHCB in Sediment from 2006 to 2019 in U.S. Receiving Water Bodies and Implications for Environmental Risk**

*Paul C. DeLeo[1], **Stella Wang**[2], Darci Ferrer[3] and Aurelia Lapczynski[4], (1) American Chemistry Council, (2) Integral Consulting Inc., (3) Fragrance Creators Association, (4) Research Institute for Fragrance Materials*

The fragrance material 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[g]-2-benzopyran (HHCB) is a polycyclic synthetic musk that, since the 1960s, has been a fragrance ingredient in many consumer and commercial products. In 2014, the U.S. Environmental Protection Agency (EPA) completed a risk assessment that indicated no concern for the use of HHCB as a fragrance ingredient in commercial and consumer products in the U.S. Despite that finding, in December 2019, EPA designated HHCB as a high-priority substance warranting risk evaluation under the Toxic Substances Control Act (TSCA). In addition, in 2021, EPA proposed designating HHCB as a persistent, bioaccumulative, and toxic chemical under the Emergency Planning and Community Right-to-Know Act. In light of these designations, we evaluated sediment monitoring data from the U.S. Geological Survey National Water Information System database. A total of 1,643 records were collected for HHCB in bottom material (sediment) collected from fresh and estuarine waterways between 2006 and 2019. In 80 percent (i.e., 1,320) of those sediment samples, concentrations of HHCB were below the limits of detection (LODs; 18–1,000 µg/kg). Though the range of LODs spans orders of magnitude, all were less than EPA's 2014 proposed concentration of concern for HHCB of 1,080 µg/kg in sediment. LODs for samples collected from the most recent 5 years of data (2015–2019; frequency of detection = 77/536) ranged from 50 to

515 µg/kg, and mean and 95th percentile upper confidence limit concentrations (with imputed nondetects based on a lognormal distribution) were 27 µg/kg and 30 µg/kg, respectively. These results for sediment supplement surface water results presented by the Fragrance Creators Association (FCA) at SETAC NA in 2021. FCA found that for surface water samples collected from 2015 to 2020, more than 60 percent of samples were nondetect for HHCB (LODs of 0.04 µg/L or 0.16 µg/L), and only two of the samples with detectable levels of HHCB had concentrations greater than EPA's surface water concentration of concern of 1 µg/L. Thus, observations from available sediment and surface water monitoring data align with EPA's 2014 analysis, and current use of HHCB in the United States does not appear to present a significant risk to organisms in the aquatic environment.

**4.20.V-05 Health significance of pesticides non-compliances of tomato and lettuce for Chilean consumers**
*Marta Riquelme-Betanzo and **Elizabeth Jara-Torres**, Universidad de Concepción, Chile*
The use of pesticides is a controversial topic because they can remain in food after their application, generating questions in the population about safety and quality in the food chain. The main objective of this work was to study the health implications for the Chilean population of tomato and lettuce samples reported as pesticide noncompliance's in the Food Information and Alert Network RIAL in 2020.

A deterministic dietary exposure analysis was performed where two exposure scenarios were considered. Vegetable consumption data proposed by the Ministry of Health MINSAL for healthy eating (Scenario 1) and data from the National Food Consumption Survey 2010, (Scenario 2) were used. Pesticide residue data were obtained from food safety events that were reported in the RIAL in 2020 for lettuce and tomato. Two types of non-compliances were considered, samples with concentrations above the Maximum Residue Limit MRL and detection of pesticides not authorized in Chile. Body weight data were obtained from the National Health Survey performed in 2010.

In 2020, 9% of the non-compliance samples were tomato and lettuce. The exposure analysis indicated that Scenario 1 (females) presents the higher exposure to pesticides. The pesticides with the highest contribution to human exposure considering Scenario 1 were chlorpyrifos-ethyl in lettuce with 26.8% of the ADI and methamidophos in tomatoes with 10.9% of the ADI. Therefore, it was possible to conclude that the notifications for the presence of unauthorized pesticide residues or residues exceeding the MRLs established for the Chilean regulation do not necessarily indicate implications for the health of the Chilean consumers. These results are relevant since the vegetables studied are the most consumed for the Chilean population being preferably used as salads without undergoing any culinary transformation process. However, it is necessary to consider that these exposure estimates are partial since they only consider the contribution of tomato and lettuce. These results may diminish the fears of those consumers who limited the consumption of vegetables, denying the positive health benefits attributed to the consumption of large amounts of vegetables in the diet.

**4.21.P Late Breaking Science: Chemistry and Exposure Assessment**

**4.21.P-Th175 High-throughput Analyses of effects of 22 per- and polyfluoroalkyl substances on Daphnia magna behavior**
***Michelle Le**, John Hoang, Monique Hazemi, Kendra Bush, Emma Stacy, Brett R. Blackwell and Daniel L. Villeneuve, U.S. Environmental Protection Agency*
Per- and polyfluoroalkyl substances (PFAS) are ubiquitously detected in the environment. However, with the exception of a few well-studied PFAS, hazard data required to evaluate risks that various PFAS pose to aquatic ecosystems are limited. The present study employed a cost-effective, high-throughput assay to screen 22 PFAS with varying chain lengths and functional groups for their effects on *Daphnia magna* behavior. Juvenile (72 h old) daphnia were exposed in a 96 well plate format to 8 concentrations of each PFAS in a ½ log dilution series. After 24 h of exposure, photomotor behavioral data was collected using video and automated object tracking

(DanioVision, Noldus). This data collection strategy takes advantage of *Daphnia magna*'s innate behavior to be stimulated by sudden changes in light intensity and swim away from light, presumably, to avoid predation from fish. Data from each individual daphnia (one daphnid per well of a 96-well plate) was exported, and statistics were processed through a custom analysis pipeline in R statistical software to evaluate effects of chemical treatment on six behavioral endpoints indicative of distance moved and activity. While the majority of the 22 PFAS tested displayed little to no behavioral effects, six PFAS significantly impacted at least three of the six behavioral endpoints. Based upon the Lowest Observed Effect Concentration (LOEC), the potency of PFAS ranked in descending order as follows: 1H,1H,8H,8H-Perfluorooctane-1,8-diol > N-Ethylperfluorooctanesulfonamide > Perfluorohexanesulfonamide > 3H-Perfluoro-2,2,4,4-tetrahydroxypentane > Perfluoroundecanoic acid > 1H,1H,10H,10H-Perfluorodecane-1,10-diol. Activity, rather than distance moved, was shown to provide the most consistent results based on the way the DanioVision instrument tracked organisms' movement. This behavioral effect assessment is being integrated into an existing daphnia high-throughput assay which will allow the comparison of behavioral effects with survival and transcriptional points of departure and shows promise to serve as phenotypic anchoring to high-throughput dose-dependent chemical exposures where LC/ECXX data alone may be insufficient. *The contents of this abstract neither constitute, nor necessarily reflect, official US EPA policy.*

**4.21.P-Th176 Equivalence of Total Petroleum Hydrocarbon Criteria Working Group (TPHCWG) and Two Dimensional Gas Chromatography (GCxGC) Analysis of Equivalent Carbon (EC) Fractions For Risk Assessment Purposes**

***Ken Scally***[1] *and Paul Nathanail*[2], *(1) Suez, United Kingdom, (2) Land Quality Management - LQM, United Kingdom*

Since the 1990s, soils and waters potentially contaminated with petroleum hydrocarbons have been analysed using a method developed by the Total Petroleum Hydrocarbon Working Group (TPHCWG) that separates hydrocarbons into aromatic and aliphatic fractions based on their equivalent carbon (EC) number. Analysis of petroleum hydrocarbons in soil or water using Two-dimensional Gas Chromatography (GCxGC) is a recent high-performance analytical technique with an increased separation capability that enhances the analysis of complex samples. GCxGC has allowed laboratories to improve the data obtained from the TPHCWG analysis, providing the risk assessor with better understanding of the nature of hydrocarbon mixtures coupled with more reliable data on EC fraction concentrations compared to those obtained from the TPHCWG method. This both provides enhanced understanding of hydrocarbon mixtures and allows laboratories to streamline their processes and deliver more consistent quality interpretative decision-making opportunities at contaminated sites. Conventional GC cannot resolve all analytes of interest, whereas GCxGC provides the risk assessor with more reliable data and increased interpretative power. The traditional turnaround times previously offered by the laboratories implementing the TPHCWG analysis are reduced significantly as the GCxGC technique eliminates the laborious sequential manual solvent extraction steps that precede one dimensional GC analysis. The traditional way is to fractionate the sample extract into its aliphatic and aromatic fractions through solid phase extraction using silica columns. The aliphatic fraction is eluted first with a suitable solvent, and the aromatic fraction is then collected in a different solvent. The extracts are passed through a florisil cleanup before GC analysis to remove any biogenic components. GCxGC involves coupling two columns with different stationary phases to allow separation of aliphatic and aromatic fractions based on two different mechanisms. The results are therefore presented in two dimensions. GCxGC has been found to be a more efficient but equivalent method of analyzing soils and waters for petroleum hydrocarbon contamination. The equivalence means risk assessors can continue to use generic assessment criteria based on the toxicological and chemical properties assigned to each fraction by the TPHCWG.

**4.21.P-Th177 Assessment of Naphthenic Acid Fraction Compounds Within an Oil Sands Pit Lake Utilizing Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry.**

***Jacob Bothen***[1], *Greg F. Slater*[1] *and Naomi L. Stock*[2], *(1) McMaster University, Canada, (3) Trent University,*

*Canada*

Oil Sand pit lakes (OSPL) are proposed to be a practicable reclamation strategy for decommissioned mine pits and the long-term storage of fluid fine tailings (FFT) in the Alberta Oil Sands Region. In 2012, Base Mine Lake (BML) was commissioned as the first large scale demonstration of OSPLs within Syncrude's Mildred Lake mining site. Recent monitoring reports are promising. However, understanding the ongoing sources and cycling of organic components within the system, particularly Petroleum Hydrocarbons (PH) and Naphthenic Acid Fraction compounds (NAFC), is important for demonstrating the effectiveness of degradation for organics as the BML system continues to develop and to help inform other planned OSPL systems. Here we report the first application of electrospray ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (ESI-FT ICR MS) to characterize NAFC, and to a lesser extent PH, within the Acid extractable organics (AEO) of water cap and FFT porewaters of the BML system. Analysis in both positive (+) and negative (-) polarity resulted in detection of an extensive array of NAFC species containing O, S, and N, as well as hydrocarbons; with a greater number of heteroatom species detected in ESI (+) mode. The distribution of AEO within BML was dominated by a narrow range of oxygenated compounds (i.e., O1-O5) and a higher abundance of S-containing compounds and was distinct from the freshwater sample from Beaver Creek Reservoir which contained less Sulfur heteroatoms and more highly oxygenated compounds more akin to natural organic matter. Within BML we observed evidence of hydrocarbon oxidation, to a greater extent within the water cap than the FFT. This included the presence of large, aromatic O1 species found only in the ESI (+) data of shallow water cap samples, suggesting bitumen or hydrocarbons found on the lakes surface are photo-oxidizing. Chemometric analysis of the data concluded that oxidized NAFC species are elevated in water cap samples which supports the occurrence of ongoing attenuation since the creation of BML, and that FFT samples are more closely associated with hydrocarbons and lower oxygenated oxy-sulfur compounds. In conclusion, ESI-FT ICR MS analysis was an effective tool for measuring AEOs and provided novel evidence supporting the ongoing degradation of PHs and NAFCs within the water cap of BML, which is a key objective for demonstrating the validity of OSPLs.

**4.21.P-Th178 Non-Targeted Identification and Semi-Quantitation of Emerging Per- and Polyfluoroalkyl Substances (PFAS) in US Rainwater Collected in the Midwest**

***Yubin Kim*** *and Paul Edmiston, The College of Wooster*

High-resolution mass spectrometry was used to screen for emerging and novel per- and polyfluorinated alkyl substances (PFAS) in precipitation samples collected in summer 2019 at seven sites in the United States. We previously quantified the concentration of ten PFAS in the rainwater samples using the method of isotopic dilution (Pike et al., 2021). Nine of these targeted analytes belonged to the U.S. Environmental Protection Agency Regional Screening Level list, herein referred to as EPA-monitored analytes. In new work, we identify emerging PFAS compounds by liquid chromatography quadrupole time-of-flight mass spectrometry. Several emerging PFAS were detected across all samples, with the most prevalent compounds being C3-C8 hydrogen-substituted perfluorocarboxylic acids (H-PFCAs) and fluorotelomer carboxylic acids (FTCAs). Concentrations of emerging PFAS were in the 10-1,000 ng $L^{-1}$ range (approximately 1-2 orders of magnitude greater than EPA-monitored PFAS) at all sites except Wooster, OH, where concentrations were even higher, with a maximum estimated ΣPFAS of 16,400 ng $L^{-1}$. The elevated levels of emerging PFAS in the Wooster samples were predominately even and odd chain-length H-PFCAs and FTCAs comprised of complex mixtures of branched isomers. This unique composition did not match any known manufactured PFAS formulation reported to date, but it could represent thermally transformed by-products emitted by a point source near the collection site. The results indicate that PFAS outside of the standard analyte lists make up a significant fraction of contaminants in rainwater collected within the central U.S.—and potentially world-wide—especially in proximity to localized point sources.

**4.21.P-Th179 Preliminary Identification and Source Tracing of Low Molecular Weight Hydrocarbons within an Oil Sands End Pit Lake**

***Han Bao** and Greg F. Slater, (1) McMaster University, Canada*
Along with significant socio-economic benefits, the extraction of bitumen from the Athabasca Oil Sands Region (AOSR) has resulted in large impacts on local landscapes. Constructing end pit lakes (EPLs) is one of the strategies to restore impacted lands and sequester fluid fine tailings (FFT). Base Mine Lake (BML), commissioned by Syncrude Ltd. in 2012, is the first commercial-scale demonstration EPL in the AOSR. Ebullition of methane from the FFT has been identified as a concern due to the contribution to atmospheric greenhouse gases, microbial oxygen consumption within the lake and ebullition-facilitated transport of organics from the FFT to the water column. The presence of low-molecular-weight hydrocarbons (LWH) thought to be derived from diluent naphtha used during bitumen extraction processes is hypothesized to be responsible for ongoing microbial methanogenesis within the FFT. The focus of this study was to identify what LWH are present within the FFT, determine their sources and assess the extent of biogeochemical cycling affecting them. Analysis involved the combination of headspace gas chromatography mass spectrometry (GC-MS) and solvent extraction comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry (GC×GC-TOFMS). Headspace analysis identified 55, 52 and 42 LWH present in the naphtha, bitumen and FFT, respectively. These were dominated by C7 to C9 *iso*- and cycloalkanes. Most compounds were present in all samples making the differentiation of naphtha versus bitumen sources of LWH challenging. However, it was noted that biodegradable LWH were absent or at low abundance in the FFT, suggesting their removal by biodegradation, likely in association with methanogenesis. Notably, C7 and C8 alkanes were observed at relatively high abundances at a site with higher methane production, consistent with the presence of LWH driving this process. Solvent extraction-based analysis via GC×GC observed a greater number of LWH present at lower abundances within naphtha and the FFT, including an array of >C9 *iso*- and cycloalkanes, also >C2-alkylated benzenes. These results indicate the most biodegradable LWH have been removed from the FFT at some sites, though they may remain at sites of high methane production. Further assessment of the biodegradability of the LWH and other organics within the FFT will help assess the potential for ongoing methane production and associated environmental impacts within the system.

**4.21.P-Th180 Assessment of Sources and Biodegradation of Naphthenic Acids in an Oil Sands Pit Lake via Multidimensional Gas Chromatography**

***Greg F. Slater**[1], Mohamed El-Waraky[1], Dave Bowman[1], Robert Nelson[2], Chris Reddy[2] and Lesley Warren[3], (1) McMaster University, Canada, (2) Woods Hole Oceanographic Institution, (3) University of Toronto, Canada*
Water capped tailing technology is currently being assessed as a reclamation approach in the Alberta Oil Sands Region (AOSR). This approach involves creation of an end pit lake by filling a mined pit with fluid fine tailings (FFT) and covering this with a water cap. The goal of this approach is the development of a functioning lake ecosystem and the concurrent sequestration of the FFT below the water cap. Syncrude Ltd. Commissioned the first oil sands end pit lake, Base Mine Lake (BML) in 2012, and has been studying its development intensively since. Recent reporting results indicate that the levels of naphthenic acids (NA), which contribute to the residual chronic toxicity of the water, have decreased within the BML water cap since commissioning, indicating that BML is effective at sequestering these compounds. Here we applied multi-dimensional gas chromatography (GCXGC) to investigate the sources and sinks of NA to the BML system in order to assess whether this sequestration can be expected to be stable.

We analyzed a set of chromatographically well resolved NA compounds that were comprised of sets of isomers of 9 NA chemical species in order to assess potential sources and sinks of NA in the system. Relative abundances of NA were highest in the deep FFT samples, indicating that the deep FFT represents an ongoing potential source of NA to the water column. In addition, observation of succinate and aromatic reaction intermediates within the FFT indicated that anerobic hydrocarbon degradation was occurring within the FFT producing additional NA. These FFT NA can be released to the water column by previously identified exchange processes including porewater advection, diffusion and methane ebullition. Within the water column, NA production was indicated via the presence of 14 unique NA that would result from the aerobic biodegradation of hydrocarbons. However, aerobic biodegradation of NA was also indicated via the presence of diacid adamantane compounds in the water column. The stability of the abundances of the NA studied here over a period of four years, while not comprehensive, indicates that despite the evidence for ongoing inputs, microbial metabolism of NA is sufficient to control NA concentrations within the surface water.

**4.21.P-Th181 How We Got to 4 ppq PFOA Quantitation in Drinking Water**

*Thep Phomsopha[1], Simon Roberts[2], Craig M. Butt[2], Karl Oetjen[2], **Matthew Noestheden**[2], Sam Lodge[3] and Andrew Patterson[1], (1) Eurofins Environment Testing (EET), (2) SCIEX, (3) Phenomenex, Inc.*

This work describes the collaboration between SCIEX and Eurofins Environment Testing to address the 2022 US EPA drinking water health advisory levels (HALs) for 4 per- and polyfluoroalkyl substances (PFAS). The aim of this project was to understand, in a proof-of-concept manner, what measures need to be taken to achieve these HALs in a commercial testing laboratory. Sample preparation and instrumental methods were developed to achieve the low parts-per-quadrillion (ppq) HALs—which is pg/L equivalent—for perfluorooctanoic acid (PFOA, 4 ppq), perfluorooctane sulfonic acid (PFOS, 20 ppq), perfluorobutane sulfonic acid (PFBS, 2 ppb) and hexafluoropropylene oxide dimer acid (HFPO-DA, or GenX, 10 ppt). The high sensitivity of the SCIEX 7500 system allowed for a simplified sample preparation procedure, reducing PFAS contamination. Method extraction spikes showed excellent recovery at 4 ppq for PFOA, PFBS and HFPO-DA, and at 20 ppq for PFOS.

**4.21.P-Th182 Online Solid-Phase Extraction (SPE) Coupled with Ultra High Performance Liquid Chromatography Tandem Mass Spectrometry (UHPLC/MS/MS) for Polyfluoroalkyl Substances (PFASs) in Human Serum Samples**

*Songmei Gao[1], Dirk Hiemstra[2], Tracey Woodruff[3], June-Soo Park[1,3] and **Sabrina Crispo Smith**[1], (1) California Department of Toxic Substances Control, (2) iChrom Solutions, (3) University of California, San Francisco*

The “forever chemicals” per- and polyfluoroalkyl substances (PFASs) are receiving widespread attention due to their environmental risks. To monitoring human exposure to those compounds, LC-MS/MS coupled with offline or online SPE methods for legacy PFAS are well established. However, a faster and sensitive analytical method for new/replacement PFAS with various structures is demanded and more challenging. In this study, a method was developed for the analysis of a broad range of PFAS compounds in human serum samples using the new generation of online extraction UHPLC/MS/MS system CHRONECT® Symbiosis Online SPE/UHPLC system (Axel Semrau®, Germany) coupled with a SCIEX Triple Quad™ API 6500$^{+}$ Mass Spectrometer (Sciex, USA). The investigated PFAS compounds included legacy PFAS and additional perfluorocarboxylic acids (PFCAs), perfluorosulfonic acids (PFSAs), fluorotelomers, ether based perfluoroalkyl acids (PFAAs) and other emerging PFASs such as Gen-X, ADONA and F-53B. Three different online SPE sorbents were evaluated for this application: CHROSPE C8 HD, CHRO SPE Phenyl and CHRO SPE Polymer DVB (Axel Semrau®). The CHROSPE C8 cartridge was employed for the validation because of its ability to accommodate more PFAS compounds. A CORTECS™ C18 column (Waters, USA) and an ACQUITY UPLC ™ BEH C18 column (Waters, USA) are used as delay column and analytical column, respectively, with a 10-minute gradient program. Among the 51 investigated PFAS compounds, the analysis of 37 compounds was successfully validated and 8 more compounds could be monitored with higher MDLs or with extra phenyl cartridges. The average recoveries of the 37 compounds range between 77.0% and 109.2% (with < 28.0% RSD, n=18). Three Proficiency Testing (PT) samples from Arctic Monitoring and Assessment Program (AMAP) were also tested using this configuration, and all 10 reported analytes passed, with z-scores between -0.69 and 0.48. Comparing to the method based on general on-line SPE HPLC/MS/MS system, the new method achieved a 2 times faster analysis time per sample and approximately an 8-fold better sensitivity, which will help to better detect thousands of PFAS potentially distributed in trace or ultra-trace levels in human body. Disclaimer: The views expressed herein are those of the authors and do not necessarily reflect those of the California Department of Toxic Substances Control. Acknowledgement: This project was partially funded by NIH/NIEHS (grant number R01ES027051)

**4.21.P-Th183 Analysis of Persistent Organic Pollutants in Sargassum from Puerto Rico**

***Emily Fritsche***[1,2] *and Emily C. Pisarski*[3]*, (1) University of Houston-Downtown, (2) NOAA EPP, (3) National Oceanic and Atmospheric Administration (NOAA)*

Macro-algal blooms of floating seaweed, *Sargassum natans* and *Sargassum fluitans (Sargassum),* have been increasing since 2011. *Sargassum* is a natural occurrence in areas from West Africa to the Gulf of Mexico, although the recent increase in blooms has become a persistent issue impacting coastal environments. This trend results in increased seaweed biomass accumulation and the expansion of the geographic range to now include the southeastern coast of the United States. It is hypothesized that as *Sargassum* mats form and sink to the bottom of these bodies of water, they accumulate pollutants and these mats may then transport accumulated chemicals across ocean boundaries. This study examined ten (10) *Sargassum* samples collected from the coast of Puerto Rico for the presence of persistent organic pollutants (POPs), including polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), polycyclic aromatic hydrocarbons (PAHs), and organochlorine pesticides using gas chromatography-mass spectrometry (GC-MS). Results of this study found that there are trace levels of POPs in all of the samples and demonstrates that *Sargassum* can serve as a vector of organic contaminants, however the environmental implications are currently unknown. The data from this study will be supplied to coastal resource managers to support best management practices and strategies for *Sargassum* mitigation.

**4.21.P-Th184 Assessment of Consumer Exposure to Emerging Chemicals in Seafood Samples from Retail Stores Around Pittsburgh Area**

***Megha Bedi***[1]*, Yelena Sapozhnikova*[2]*, Raegyn B Taylor*[2] *and Carla A. Ng*[1]*, (1) University of Pittsburgh, (2) U.S. Department of Agriculture*

Seafood, including fish and shellfish, is a major source of omega-3 fatty acids crucial for heart health, and intake trends show an increasing percentage of people consuming seafood worldwide, including the US. Increasing seafood demand has led to a surge of products on the market including those labelled as sustainable, imported, locally-caught, and farmed. Concurrently, seafood has emerged as a potential source for exposure to many chemical contaminants, but a critical factor that remains largely unknown is whether consumption patterns translate to differences in chemical exposures. Here, we monitor a wide suite of chemicals including veterinary drugs, pesticides, and environmental contaminants including polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs), and per- and polyfluoroalkyl substances (PFAS) in seafood samples collected from retail stores in the Pittsburgh PA area. A total of 46 seafood samples were screened for 450 compounds across three mass spectrometry systems: 286 by ultra-high-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS), 252 by low-pressure gas chromatography- tandem mass spectrometry (LPGC-MS/MS) in which 93 analytes overlapped with UHPLC, plus 33 PFAS by UHPLC-high-resolution mass spectrometry (HRMS; Orbitrap). Measured concentrations will be compared with maximum residue levels (MRLs) where established and used to build exposure estimates based on diet scenarios (e.g., cost, cultural preferences, and origins). This study will help investigate whether commercially available seafood is safe for human consumption and if exposures depend on consumption patterns.

**4.21.P-Th185 Development and evaluation of a heavy metals speciation and transport model in the multimedia environment**

***Ja Eun Jung***[1]*, Hee Seok Kim*[2]*, Jihyun Park*[1] *and Dong Soo Lee*[1]*, (1) Seoul National University, Korea, Republic of (South), (2) Seokyeong University, Korea, Republic of (South)*

A heavy metal (HM) can exist in multiple forms (species) in the environment. For more accurate and reasonable risk assessments, it is necessary to predict the concentrations of individual species of the HM as the fate and toxicity depend on the forms. For environmental modeling purposes, HMs may be divided into three groups, i.e., Group I at a single oxidation state (Al, Co, Ni, Cu, Zn, and Pb), Group II with redox process (Cr, Mn, Fe, and Cu), and Group III involving many complex speciation processes (As, Se, and Hg). In this study, we developed a multimedia model for HMs in the Groups I and II. In the model, HMs speciation is accounted for by equilibrium and kinetic approaches. With the equilibrium approach, partitioning is calculated using a partition coefficient estimated as a function of pH, soil organic matter and/or the total HM concentration. Three forms (free ion, DOM-metal, and inorganics-metal complexes) are proposed in the dissolved phase and their distribution is estimated by V.MINTEQ. With the kinetic approach, redox processes are modeled based on the relationship between oxidation and reduction rates. The model can estimate concentrations of particulate and the three dissolved phase species (for both the oxidized and reduced HMs in Group II). The predicted concentrations in water were compared against measurements obtained in South Korea. The ratios ($C_{predicted}/C_{mesured}$) based on the average total concentration in water are 0.24 (Al), 0.28 (Co), 0.29 (Ni), 2.67 (Pb), and 0.32 (Zn) in Group I, and 4.20 (Cr), 1.02 (Cu), 0.10 (Fe), and 1.05 (Mn) in Group II, indicating generally underestimation by the model. However, most of the predictions (99.9% (Al), 99.9% (Ni), 97.6% (Pb), and 100% (Zn)in Group I, and 100% (Cr), 100% (Cu), 72% (Fe), and 100% (Mn) in Group II) fell within the range of the measured concentrations. The predicted concentration fractions of the dissolved and the particulate Zn were 0.56 (±0.27) and 0.44 (±0.26), respectively while those of the measurements were 0.74 (±0.24) and 0.25 (±0.24), respectively. The estimated concentration fractions of the dissolved $Cr^{III}$ and $Cr^{VI}$, and the particulate Cr were 0.29 (±0.04), 0.13 (±0.12), and 0.58 (±0.08), respectively, as compared to the measured values of 0.24 (±0.28) for dissolved $Cr^{VI}$. While further evaluation is needed, these demonstrate the feasibility of the model to be used for risk assessments accounting for HMs speciation.

**4.21.P-Th186 Building towards the development of nanoplastic controls: Investigating the chemical signatures of pure polymer, size-dependent plastic degradation through multiple pathways**

***John M. Pettibone*** *and Thomas P. Forbes, National Institute of Standards and Technology (NIST)*

Detection and chemical identification of small microplastic and nanoplastic particles (MNP) collected and isolated in field campaigns have remained a significant analytical challenge, which is further complicated because chemical signature changes induced by the same degradation mechanism can be compositionally (or by polymer class) distinct. Identifying chemically modified MNPs in the < 5 µm size regime using field standard spectroscopic tools, FTIR and Raman, and mass spectrometric techniques is challenging because libraries used for spectral matching in both instances are incomplete. An understanding of how chemical modification (e.g., oxidation from thermal, photo, biotic, etc.) as a function of composition, size and morphology affects spectroscopic and mass spectrometric spectral features would lower uncertainty for identification, enable improved quantification, and ultimately improve understanding of fate for plastics in engineered and environmental matrices. To begin addressing uncertainty associated with chemical identification, we investigated the size dependent oxidation of two plastics controls, polyethylene terephthalate (PET) and polypropylene (PP), which were cryomilled from pure, well-characterized nurdles and processed under several different oxidation pathways. Using a suite of spectroscopic and spectrometric analytical tools, the preliminary comparisons of the chemical signatures for the two compositions at different sizes, including their bulk counterparts, will be presented. This analysis will provide direction for future work aimed at identifying candidate, "aged" MNP controls.

**4.21.P-Th187 A Data Visualization Dashboard for Microplastics Environmental Monitoring Data**

***Todd Gouin***[1]*, John Norman*[2] *and Richard A. Becker*[2]*, (1) TG Environmental Research, United Kingdom, (2) American Chemistry Council*

Concern over microplastics in the environment has emerged as a global issue in recent years. As microplastics environmental monitoring studies proliferate, scientists and decision makers need better tools to access, visualize and analyze this monitoring data. To better understand and facilitate access to published microplastics environmental monitoring studies and data, a pilot project was initiated by the American Chemistry Council's Long-range Research Initiative (ACC LRI) to collect and organize published studies using a data visualization tool. To develop the dashboard, an available dataset of published studies (through 2019) reporting measurements of microplastics in several environmental media (biological, sediment, soil, and surface water) was reviewed and relevant data were extracted and cataloged. The data (extracted from 587 references) were then linked to the Tableau® analytics platform to be visualized and queried interactively. This dashboard (at tinyurl.com/4acd4knk) permits filtering and visualization of attributes such as sample type (air, water, sediment, etc.), collection location, polymer type, particle shape, etc. The dashboard can readily sort, or drill to a data element or hierarchies, and users can then download these sets of selected data and references, including data on particle sizes. The dashboard, which includes instructions for use and a link to provide feedback and suggestions for improvement, is now freely available as a beta version. We encourage all who are interested in microplastics research to test drive the dashboard and provide feedback over the next six months. This pilot project will then be evaluated and a decision will be made whether to further develop and update the dashboard with more recent studies.

**4.21.P-Th188 Occurrence of PFAS and PFOS in Surface Waters of a UNESCO World Heritage Site in Brazil**

***Maria Clara Starling*[1]*, Camila Amorim*[1]*, Matt Simcik*[2] *and Daniel A.S. Rodrigues*[1]*, (1) Universidade Federal de Minas Gerais, Brazil, (2) University of Minnesota*

Per- and polyfluoroalkyl chemicals (PFAS) have been produced and used since the 1940's. As their chemical structure contains at least one perfluoralkyl bond ($C_n$-$F_{2n}$), they are hard to degrade and persist in the environment, thus being referred to as "forever chemicals". In addition, they are known to accumulate in the food chain and have been associated to lower immune response as well as kidney and liver cancer. Although some of the most well-known PFAS, such as PFAS and PFOS, have been regulated, there are currently more than 4,000 thousand PFAS in use, most still unregulated. Recent studies have revealed PFAS occurrence in environmental compartments (surface water, groundwater wastewater, sediments, soil and atmosphere) in developed countries, leading to discussions towards the adoption of environmental criteria for aquatic life and human health protection. Despite efforts to untangle PFAS contamination in developed countries, only a few studies have been conducted in developing regions. Hence, it is important to investigate PFAS contamination in these regions. The aim of this study was to assess the occurrence of PFAS in surface water from an urban lake located in Belo Horizonte, MG, Brazil. Pampulha Lake and its surrounding area were given the title of a UNESCO World Heritage Site in 2016. Despite that, the lake still receives raw sewage from neighborhoods within the watershed and which are not connected to the sewer system. Sampling was performed from March to August 2022 in four sampling points located in different regions of the lake. Sample preparation and analysis were performed for 24 PFAS as according to the draft EPA 1633 Method. Results revealed the occurrence of PFOA and PFDA in all sampling points in concentrations ranging from 10 to 60 ppt. PFBS, PFOS, PSHxS and PFDoA were also detected in some samples. Detected concentrations are more than 3-fold higher than drinking water safety values recommended by the EPA for PFOS (0.02 ppt) and PFOA (0.004 ppt). Although surface water from Pampulha lake cannot be used for drinking water supply, PFAS contamination levels in the lake are concerning as illegal fishing for human consumption takes place in the lake.

**4.21.P-Th189 Suspect and Non-target Screening of Chemicals in Consumer Cleaning Products**

***Kyung-Duk Zoh****, Jeonghoon Park and Hyejin Yun, Seoul National University, Korea, Republic of (South)*

The increasing use of consumer products made of numerous chemical substances increases the risk of exposure to humans through various environmental routes. To reduce the risk of exposure to daily consumer products and to evaluate the behavior of substances contained in the consumer products in the environment and their adverse effects to humans, it is essential to identify the substances in consumer products. In this study, 31 consumer cleaning products, including 10 types of cleaning products (bathroom/toilet, kitchen, glass, metal, air conditioner, automobile, carpet, multipurpose, floor, and bowling ball), were examined to identify substances contained in the products, and resulting their toxicities were evaluated. After instrument analysis of samples for substance detection using LC-QTOF-MS, suspect and non-target screening were performed using UNIFI software. The substances detected with high frequency were plasticizers (freq. 74.2%), surfactants (freq. 71.0%), and antioxidants (freq. 64.5%). A total of 49 substances were identified with high frequency in the suspect screening. In the results of non-target analysis, total 51 substances were identified. Amomg them, oleamide, a type of surfactant, was confirmed at a high frequency of 74.2%, probably due to the structural characteristics of amide containing 6 chemical substances. The ECHA database was used to evaluate the toxicity of the 100 substances (suspect: 49, non-target: 51) identified in this study. /21 substances (methyl 1,2,2,6,6-pentamethyl-4-piperidinyl sebacate, myricetin, azithromycin, pentaerythritol triallyl ether, lomefloxacin, methyl tetrahydrophthalic anhydride, linalool oxide, 1,4-butanediol diglycidyl ether, octabenzone, (.+-.)-limonene, (9Z)-N-methyl-9-octadecenamide, diethyl caprylamide, diisopropyl methylphosphonate, elaidamide, isostearamidopropyl dimethylamine, lauramide, lauramidopropyl dimethylamine, methyl(trifluoromethyl)dioxirane, tetradecanamide, oleamide, palmitamidopropyl dimethylamine) were classified as a substance of caution. The 21 substances classified as cautionary substances that were identified in both toxicity assessment tools have a high level of toxicity.

### 4.21.P-Th190 Occurrence and Distribution of Eight Canadian Approved Ultraviolet Filters in Outdoor Swimming Pools and a Public Beach on the Canadian Prairies

***Alistair Kendrick Brown**, Annemieke Farenhorst, Jade Wish and Tori Maxwell, University of Manitoba, Canada*

UV Filters are found in a wide range of cosmetics including sunscreens. Some are resistant to sunlight by design, and others purposefully breakdown to protect human health. UV filters are known to “bleach” coral and be toxic to aquatic organisms in both freshwater and marine environments. They are also known to be strong endocrine disruptors and estrogen mimics. Currently, there is very little freshwater data in Canada. We quantified eight of the most popular and common UV filters approved for Canadian usage in three outdoor swimming pools in Winnipeg, Canada and a nearby freshwater beach over the 2022 summer season. The chemicals were avobenzone, dioxybenzone, homosalate, octisalate, octinoxate, octocrylene, oxybenzone, and sulisobenzone. This was done using solid phase extraction coupled with polarity switching via ultra-high performance liquid chromatography-tandem mass spectrometry. We sampled weekly to establish seasonal trends of usage, every day for one week to determine UV filter persistence and build up/ transformation in the short-term, and every hour over one day to elucidate these same trends in the shortest term. In chlorinated outdoor pools there was minimal water renewal, ultraviolet disinfection, and high public usage over the entire season. We saw a consistent trend of dioxybenzone, oxybenzone, and sulisobenzone mean levels of -1, avobenzone, homosalate, octisalate, and octinoxate mean levels of 0.009 to 0.25 $\mu g\ L^{-1}$, and mean octocrylene levels ranging from 1.52 to 2.49 $\mu g\ L^{-1}$. In the mesotrophic freshwater beach environment the range of mean concentrations were avobenzone (0.050 $\mu g\ L^{-1}$) , dioxybenzone (0.031 $\mu g\ L^{-1}$), homosalate (0.089 $\mu g\ L^{-1}$), octisalate (0.038 $\mu g\ L^{-1}$), octinoxate (0.021 $\mu g\ L^{-1}$), octocrylene (0.034 $\mu g\ L^{-1}$), oxybenzone (0.16 $\mu g\ L^{-1}$), and sulisobenzone (0.017 $\mu g\ L^{-1}$). The differences between pools and beach are most likely due to much greater natural dilution of the lake and lack of chlorine ions to contribute to transformation. Also, the absence of soil, sediment, and suspended organic matter in the pools, in addition to the forced recycling of the water promoted their suspension in the pool water regardless of what their fugacities were. This was especially evident in octocrylene which is a very oily substance (log Kow= 6.9) having persistently high concentrations in all pools all season. These methods and findings can be used to assess whole organism toxicity, and establish water quality guidelines.

### 4.21.P-Th191 Diffusive Fluxes of Persistent Organic Pollutants Between Arctic Atmosphere, Surface Waters and Sediments

***Ian Moran**[1], Lane Tidwell[1], Michael Barton[1], Molly Kile[1], Pamela Miller[2], Diana Rohlman[1], Samarys Seguinot Medina[2], Bobby Ungwiluk[2], Viola Waghiyi[2] and Kim Anderson[1], (1) Oregon State University, (2) Alaska Community Action on Toxics*

Arctic communities are disproportionately exposed to persistent organic pollutants from sources including global atmospheric transport and formerly used defense sites (FUDS). The effects of global climate change and increasing development in the Arctic have the potential to exacerbate this problem. Yupik residents of St

Lawrence Island, Alaska are one such community with documented exposures to pollutants from FUDS, and traditional foods like blubber and rendered oils of marine mammals. Troutman Lake, adjacent to the Yupik village of Gambell, Alaska, was used as a disposal site during the decommission of the adjacent FUDS, leading to community concern about exposure to legacy military pollution. In collaboration with a local community group, this study utilized passive sampling devices deployed at eight locations in Troutman Lake. Air, water and sediment pore-water deployed samplers were analyzed for 41 unsubstituted and 22 alkylated polycyclic aromatic hydrocarbons (PAHs), 43 brominated and organophosphate flame retardants and 52 polychlorinated biphenyls (PCBs). Sum PAH concentrations ranged from 20 – 55 ng/$m^3$ in air, 1.4 – 3.2 ng/L in surface water and 0.46 – 1.4 ng/L in sediment pore-water. Naphthalene was the most abundant PAH in the air and water, constituting more than 70% of total PAHs. In the sediment pore-water, PAHs were primarily three ring compounds, in particular, phenanthrene and sulfur heterocycle dibenzothiophene. The majority of PAHs were in deposition from the overlying atmosphere into Troutman Lake. Brominated diphenyl ether 47 was detected in all surface water samplers (0.0018 – 0.0156 ng/L). Organophosphate flame retardant, triphenyl phosphate was detected in all environmental compartments at concentrations less than 0.004 ng/$m^3$, 0.07 ng/L and 0.07ng/L in air, water and sediment porewater, respectively. Of particular interest, we measured higher vapor phase concentrations of tris(2-chloroethyl) phosphate (TCEP)(0.75 - 2.8 ng/$m^3$) than previously reported in the literature for similar remote Arctic sites (0.017 – 0.56 ng/ $m^3$). TCEP was found to be in deposition to Troutman Lake at magnitudes ranging from 290 ng/$m^2$/day to 1300 ng/$m^2$/day. No PCBs were detected in this study. These findings can help us to understand the fate of anthropogenic contaminants in dynamic Arctic systems with both global and local sources of modern and legacy pollutants.

**4.21.P-Th192 Extent of Neonicotinoid and Fipronil Contamination in Minnesota Hydrogeologic Systems**
***Grant Goedjen**, Matthew Berens and William Arnold, University of Minnesota*
The use of neonicotinoid's and Fipronil have become ubiquitous in midwestern agriculturally-dominated regions for the elimination of pest species from crop fields with approximately 90,000 lbs of pure product applied in 2020 alone. Their presence in seed coatings, spray treatments, and soil injections allow for the formation of pesticide-latent surface runoff from agricultural fields for transport into surface and subsurface hydrologic systems; several neonicotinoid's and fipronil have been observed in midwestern lakes and streams at appreciable concentrations in the ng/L-µg/L concentration range. Few studies have been conducted evaluating the extent of pesticide contamination in hydrogeologic systems recharged by primarily agricultural regions which provide drinking water to several metropolitan areas, municipalities, and thousands of private wells. To better understand the risk associated with groundwater and spring resource extraction with respect to pesticide contamination, 129 samples were collected from 16 superficial springs and 79 groundwater wells over a four-year period in urban and rural settings to evaluate the extend of contamination in Minnesota's groundwater systems. Thiamethoxam was the most commonly observed neonicotinoid detected in 44% of superficial springs and 11% of groundwater wells. Imidacloprid (27%, 12%), clothianidin (35%, 8%), and acetamiprid (28%, 5%) were detected in springs and groundwater wells at similar rates with thiacloprid occurring in only 19% of springs and 16% of wells sampled. Total neonicotinoid concentrations were observed as high as 199 ng/L for clothianidin in springs and 32.6 ng/L in groundwater. Superficial springs were significantly ($p<0.001$) more likely to have pesticide contamination with a detection rate 2-3 times higher and observed concentrations orders of magnitude larger than their deeper groundwater counterparts. Highly concentration samples for clothianidin, acetamiprid, and thiacloprid were contained predominantly to surface springs while acetamiprid was observed as low as 100 ft and thiacloprid and fipronil observed as low as 200 ft below the surface in groundwater systems. Concentrations of clothianidin, imidacloprid, and thiacloprid were consistently observed at higher concentrations in areas with high proportions of agricultural and undeveloped regions while fipronl was observed at highest concentrations in agricultural regions.

**4.21.P-Th193 Global Occurrence and Water Quality Hazards of Synthetic Glucocorticoids in Wastewater Effluent and Surface Waters**

*__Alexander R. Cole__ and Bryan W. Brooks, Baylor University*

With growing urban population density across the globe comes the increase in pharmaceutical usage. The most rapid expansion is currently in economically disadvantaged nations, where wastewater treatment and other chemical and waste management activities are often limited or nonexistent. We conducted a global scanning assessment of synthetic glucocorticoids in wastewater effluent and freshwater systems. Thirty-eight synthetic glucocorticoids were identified, and a critical review of available literature was performed. Subsequently, we developed environmental exposure distributions (EEDs) for synthetic glucocorticoids, and further considered glucocorticoid receptor agonistic activity measured via in vitro bioassay methods. When sufficient data was available, we then performed probabilistic environmental hazard assessments (PEHAs) using predicted non-effect concentrations (PNECs), therapeutic hazard values (THVs) and in vitro bioactivity information for specific glucocorticoids gathered from EPA's CompTox Chemicals Dashboard. Synthetic glucocorticoid occurrence was ubiquitous in Africa, Asia, Europe, North America, Oceania and South America. However, data were minimal for Africa, western Asia, Oceania and a majority of the Americas. Likewise, occurrence of glucocorticoid receptor agonism was prevalent in Europe, North America, and Oceania, with a vast majority of data coming from the United States of America. In addition, we critically examined aquatic toxicology information for these substances and subsequently identified a lack of sub lethal toxicity data for non-target species across compounds. These critical reviews and meta-analysis elucidate important data gaps in our current understanding of synthetic glucocorticoids in the environment and promises to inform environmentally relevant studies in the future.

**4.21.P-Th194 Pharmaceutical Bioaccumulation and Disposition in Fish across Trophic Positions**

*__Jaylen Lesean Sims__, Alexander R. Cole, Zachary S. Moran, Charles Mansfield, Bianca L. Possamai, Macarena Gisele Rojo, Ryan King, Cole W. Matson and Bryan W. Brooks, Baylor University*

Bioaccumulation of pharmaceuticals by aquatic organisms continues to receive attention by the scientific community. However, the relative disposition of these contaminants among different tissue compartments of fish species has received much less investigated, particularly among trophic positions. Herein, biological based read-across may be useful to predict contaminant disposition within fish. Herein, we examined understanding fish from the Brazos River in Waco, Texas, USA, which is influenced by effluent discharge from a wastewater treatment plant (WWTP). Along with water samples, longnose gar (*Lepisosteus osseus;* piscivore), gizzard shad (*Dorosoma cepedianum*; planktivore/detritivore), and smallmouth buffalo (*Ictiobus bubalus*; benthivore), were collected and dissected for their plasma, brain, gills, gonads, liver, and lateral muscle fillet. Water, plasma, and fish tissues were analyzed via isotope dilution liquid chromatography tandem mass spectrometry. In addition, trophic position was determined using isotope ratio mass spectrometry. WWTP tracers, caffeine and sucralose were found at low µg/L levels in surface water, while an anticonvulsant, carbamazepine, was up to 37 ng/L. The selective serotonin reuptake inhibitors (SSRIs) fluoxetine and sertraline and their primary metabolites were detected in at least one tissue of all three species at low µg/kg concentrations. Within each species, brain and liver of select fish species contained the highest levels of the SSRIs compared to the plasma and other investigated tissues, which is generally consistent with our initiation reports of pharmaceutical accumulation in other species that generally paralleled human disposition patterns. However, we observed accumulation differences among specific tissue types and species. For example, mean levels of sertraline in brain tissue were 13.4 µg/kg in gizzard shad and 1.1 µg/kg in longnose gar, respectively, and mean liver concentrations were 1.5 µg/kg in gizzard shad and 6.8 µg/kg in longnose gar, respectively. In contrast, smallmouth buffalo did not consistently accumulate SSRIs to detectable levels. Future efforts are necessary to understand such marked bioaccumulation and internal dispositional differences among freshwater fish species occupying different trophic positions.

**4.21.P-Th195 Per- and Polyfluoroalkyl Substances in Firefighter Turnout Gear Following Exposure to Abrasion, Heat, Laundering, and Ultraviolet Radiation**

*__Andrew Maizel__[1], Andre Thompson[2], Meghanne Tighe[2], Samuel Escobar-Veras[2], Alix E. Rodowa[2], Ryan*

*Falkenstein-Smith[2], Bruce Benner[2], Michelle Donnelly[2], Benjamin Place[2], Jessica Lynn Reiner[2], John R. Kucklick[2] and Rick Davis[2], (1) Georgetown University, (2) National Institute of Standards and Technology (NIST)*
Firefighter turnout gear is typically composed of layers that include both fluoropolymer membranes as well as fluoropolymer-treated textiles and has recently been identified as a potential source of per- and polyfluoroalkyl substance (PFAS) exposure to firefighters. Additionally, higher PFAS concentrations have been observed in used compared with unused turnout gear, though the extent to which fluoropolymer degradation and PFAS accumulation from firefighting activities contribute to this difference is still being investigated. To evaluate whether typical use impacts turnout gear PFAS concentrations, this study quantified over fifty PFAS, including perfluoroalkyl acids as well as sulfonamide-containing and fluorotelomerization-derived PFAS, in twenty-one turnout gear textiles before and after exposure to abrasion, heat, laundering, or ultraviolet (UV) radiation. Twenty-six individual PFAS were quantified above their reporting limits in unused turnout gear textiles, with higher total PFAS concentrations observed in unused outer shell textiles compared with moisture barrier or thermal liner textiles. Total PFAS concentrations in outer shell textiles increased by two to eleven times with exposure to abrasion, heat, or UV radiation but were largely unchanged by laundering. Conversely, total PFAS concentrations in moisture barrier and thermal liner textiles showed no consistent variation with any of the examined stressing processes. 6:2 fluorotelomer methacrylate and 6:2 fluorotelomer alcohol were measured at the highest concentrations of any PFAS in both unused and stressed textiles, suggesting that remnant materials from side-chain fluorinated acrylate polymer production were the largest source of PFAS. While exposure to stresses associated with typical use did increase measured PFAS concentrations in some textiles, variation within each textile type suggests that the total amount of the PFAS present may vary by up to a factor of six according to the specific textiles selected for turnout gear construction. Accounting for PFAS concentrations while choosing turnout gear textiles could therefore potentially reduce firefighter exposure to PFAS as well as reduce the environmental release of PFAS when turnout gear is washed or disposed.

**4.21.P-Th196 Optimizing a Pesticide Fate Model for Use in Predicting Honeybee Risk under Different Crop and Weather Scenarios**
*Ashlie Kinross[1], **Kimberly J. Hageman**[1], Sean Lyons[1], Micah McClure[2] and Randa Jabbour[2], (1) Utah State University, (2) University of Wyoming*
Tools are needed to better predict and understand pesticide risks to pollinators under different scenarios. Pesticide fate modeling can be used to predict pesticide concentrations in field matrices after application. However, pesticide-specific foliar photodegradation rates are needed as input values in these models and few such values exist in the literature. In this work, we measured the foliar photodegradation rates of three insecticides (chlorpyrifos, λ-cyhalothrin, and indoxacarb), both as the active ingredients alone and in commercial formulations, on alfalfa leaves and incorporated these values into the Pesticide Dissipation from Agricultural Land (PeDAL) model. We then conducted six case studies in which chlorpyrifos and λ-cyhalothrin were applied to alfalfa fields under varying crop and weather conditions, and dissipation rates were measured. A comparison of measured and modeled concentrations in leaves over seven days following application indicated that leaf penetration rates varied between insecticides and needed optimized in the model. Following optimization, measured and modeled concentrations in leaves agreed well. Honeybee risk assessments were then conducted for each scenario by estimating pesticide exposure from oral and contact routes and then determining risk quotients (RQs) based on lethal doses. The wait periods needed for pesticide concentrations to drop to safe concentrations for honeybees (i.e. when the RQs were < 1) were then determined for each case scenario. For chlorpyrifos, the concentrations were above the safe levels even after seven days. For λ-cyhalothrin, the safe levels were reached between 5.5 and >7 days, depending on field conditions. The value of this work is that we demonstrate how pesticide fate modeling can be used in combination with toxicity endpoints to predict time periods needed to reduce insecticide effects to desired levels, and that these time periods vary with crop and weather conditions. The same approach can also be used to predict insecticide

efficacy times against pest insects, and better understand insecticide resistance, thus aiding Integrated Pest Management efforts. A web-based application for use by the general public will also be introduced.

**4.21.P-Th197 Developing a Research Framework Aimed at the Translation of in vitro High Throughput Screening Data for Environmental Exposures Assessments.**

***Christopher Andrew James***[1], *Ruth M. Sofield*[2] *and Maya Faber*[2], *(1) University of Washington, (2) Western Washington University*

High throughput *in vitro* approaches provide exposure-response bioactivity information based on laboratory exposures of cell lines, receptor models, or proteins. HTS data are being generated via more than 15 federal government (the Tox21 program) and commercial platform services that characterize responses for over 2100 assays covering approximately 300 signaling pathways. Tox21 has screened ~8500 compounds with more than 70 different high-throughput assays. Program data are compiled in the US EPA CompTox chemical database.

The time and expense of completing an *in vivo* assay limits the acquisition of toxicity information. *In vitro* assays can be completed in a shorter time and provide empirical information on chemical interactions with biological systems. Additionally, many governments are phasing out the use of animal models indicating that HTS approaches will be increasingly relied upon to provide information that may not otherwise be available. As such, there is value in expanding its applicability to environmental systems.

There are recognized challenges to evaluations based on HTS data. Much of it is obtained utilizing mammalian cell lines, receptor models, and sequences, thus it is necessary to consider whether the domain of applicability includes non-mammalian species. Additionally, it has not been established how well the cellular and biochemical endpoints predict apical endpoints, although some work using Adverse Outcome Pathways (AOPs) have been used to link these effects. Finally, translating exposures from *in vitro* experimentation to environmental exposures is complex and requires consideration of chemical properties, such a partitioning and susceptibility to degradation, and toxicokinetics. These factors should be included as part of the derivation of translational exposure-response threshold values for environmental systems.

By way of this poster, we are interested in polling the community of practice to augment and highlight conceptual models, research needs, and data gaps for the effective translation of HTS information to environmental systems. Feedback will be collected through discussion and input via a QR code. Findings may then be described in a broadly-collaborative publication.

**4.21.P-Th198 The Soil-Water Partitioning Behavior of Oxyfluorfen Under California Rice Field Conditions**

***David Joseph Bonnar***, *Jeffry Patrick Eichler, Sanjai Parikh, Matt Hengel and Ronald Tjeerdema, University of California, Davis*

Oxyfluorfen (trade name Goal) is a broad-spectrum, diphenyl ether herbicide used for pre- and post-emergent control of a variety of broadleaf and grassy weeds. Although registered over 40 years ago, the recent discovery of non-transgenic, oxyfluorfen-resistant rice strains in addition to demonstrated effectiveness on rice weeds has sparked a surge in interest, with researchers and herbicide manufacturers racing to bring a rice-field approved product to market. This process, however, faces the challenge that oxyfluorfen has historically been prohibited from use in or near aquatic sites due to its high toxicity to aquatic organisms. When rice fields are treated with herbicides, the potential exists for their discharge into receiving waters, risking exposure to wildlife, drinking water contamination, and transport to other agricultural fields. Thus, it is imperative that any decisions regarding registration of oxyfluorfen products for use in rice fields be supported by rice field-relevant fate data. In this study, the batch equilibrium method was used to characterize two of the most widely used parameters in predicting the distribution and fate of herbicides in the environment, the soil-water and organic-water partitioning coefficients, $K_d$ and $K_{oc}$. These properties were examined in two California rice field soils and under simulated California rice field conditions (e.g., temperature, saline environment). Both sorption and desorption processes were analyzed and isotherms constructed to evaluate potential binding mechanisms and degree of reversibility. We show that oxyfluorfen exhibits ideal, C-curve linear sorption behavior with a tendency to sorb both strongly and proportionally at a variety of initial concentrations. Desorption was also highly proportional to amount sorbed, indicating the sorption was reversible and oxyfluorfen primarily sorbed to the surface of soil organic matter with limited ability to penetrate its matrix. These patterns were consistent across a range of environmentally relevant conditions, indicating that oxyfluorfen can be expected to be found predominantly in the soil, yet still retain the potential for gradual release from soil into water when applied as an herbicide to rice fields.

## 4.21.V Late Breaking Science: Chemistry and Exposure Assessment

### 4.21.V-01 Development of Sample Preparation Methods for Chemical Analysis of Household Chemical Products

***Hyeonjeon Cha**, Woo-Young Song and Tae-Young Kim, Gwangju Institute of Science and Technology, Korea, Republic of (South)*

Although most household chemical products (HCPs) are generally safe, chemicals in HCPs may cause unexpected adverse effects on the human body if they are not correctly stored or used. Establishment of reliable sample preparation methods tailored to cover a wide range of HCPs is a prerequisite for evaluation of the potential toxicity of HCPs. In this study, we developed sampling and sample preparation methods for liquid chromatography tandem mass spectrometry (LC-MS/MS) to comprehensively identify the ingredients in 20 HCPs having diverse forms and formulations. Solid phase extraction of mid-polar to non-polar analytes with a basic solvent significantly decreased matrix interferences during electrospray ionization, leading to increased numbers of compound identification. Untargeted LC-MS/MS of 20 HCPs could tentatively identified 134 compounds using the NIST 20 MS library. Functional annotation of the identified compounds based on PubChem and CompTox database searches revealed that erucamide and alpha-ionone that are highly relevant to HCPs have the potential to cause skin irritation. Our developed sample preparation method for untargeted LC-MS/MS can be exploited to discover potential toxicants in HCPs.

### 4.21.V-02 Predicting appropriate extraction methods for a variety of PFAS in different matrices based on their physical-chemical properties

***Luciana Cappelini**[1], Vinícius Ricardo Acquaro Jr.[2] and Natalia Soares Quinete[1], (1) Florida International University, (2) Scientia Laboratório de Pesquisa, Desenvolvimento e Inovação LTDA, Brazil*

Per and polyfluoroalkyl substances (PFAS) are synthetic substances with physicochemical properties that repel water and oil efficiently. These "non-stick" qualities have made PFAS used in many industrial processes and commercial products, which has resulted in its widespread presence in several environmental compartments such as surface water, drinking water, sediments, soil, food, and biota, including in humans. Ecotoxicological assays to measure PFAS effects on aquatic organisms are usually performed using zebrafish (*Danio rerio*) and have been mostly focused on long-chain compounds toxicity. With the increasing spotlight on short-chain PFAS, studies are needed to address the toxicity of many PFAS that have not yet been studied. In addition, a comprehensive methodology for "total" PFAS extraction from a variety of matrices, including water, biota, and soil, still needs to be developed and optimized, considering the wide range of physicochemical properties of these compounds. Most target PFAS approaches are performed by solid phase extraction (SPE) using a weak anionic exchange cartridge or column followed by liquid chromatography-mass spectrometry analysis, which has shown good performance for some groups of anionic PFAS but does not apply to cationic and zwitterionic PFAS. The diversity of PFAS physical-chemical properties limits the application of a unique sample preparation technique to extract all molecules in different matrices. In this work, we will use the Percepta software, a platform for in silico molecular properties calculations, to group PFAS with similar properties and predict the appropriate extraction techniques for a variety of PFAS classes in aqueous and solid matrices based on their physical-chemical properties. The software predicts PFAS properties such as pka, Log P, Log D, solubility, acute and aquatic toxicity, and mutagenicity from molecular structures. PFAS will be also separated into groups based on their toxicity levels. The main idea is to identify among the EPA PFAS Master list those compounds with the greatest toxicological concern. For the extraction, one of the main drivers to establishing an efficient and robust sample preparation is understanding the interactions between the molecules, which are highly dependable on each PFAS functional group. Based on that, protocols for sample extraction will be defined using sample preparation techniques, such as Solid Phase Extraction (SPE) and Solvent Extraction (SE).

**4.21.V-03 Aerial Transport of Pyrethroids and Avermectins via Wind-blown Particulate Matter >12 km Downwind of Beef Cattle Feedlots**

***Amanda Emert**, Frank B. Green and Philip Smith, Texas Tech University*

Beef cattle feedlot-derived particulate matter (PM) has been identified as a source of complex mixtures of pesticides, veterinary pharmaceuticals, and bioaerosols. However, no literature currently exists quantifying insecticide and anthelmintic transport via wind-blown PM beyond 1 km. In the current study, downwind transport of PM from three feedlots (animal unit capacity = 75,000-82,500) in the Southern Great Plains of North America was investigated. High-volume samplers collected total suspended particulates (TSP) in 30 min. intervals at one upwind (≤0.5 km) and three downwind locations (D1, ≤1.1 km; D2, 2.0 - 5.9 km; D3, 7.3 – 12.4 km) over six evenings per feedlot (n = 18). Subsequently, TSP samples were extracted and analyzed via UHPLC-MS/MS for six pyrethroid insecticides (bifenthrin, λ-cyhalothrin, cypermethrin, esfenvalerate, fenvalerate, and permethrin) and five avermectins (abamectin, doramectin, eprinomectin, ivermectin, and moxidectin). Statistical analyses of log-transformed TSP concentrations and log($x$+1)-transformed analyte concentrations were performed via linear mixed models (LMM) for repeated measures and post hoc Bonferroni adjustment for multiple comparisons. Significantly elevated TSP concentrations were observed at D1-D3 (grand mean ± SE (μg/$m^3$); D1 = 16,996 ±4,437, D2 = 2,776.8 ±392.3, D3 = 1,484 ±239.8) relative to upwind locations (all, p <0.001; grand mean = 251.2 ±72.4 ug/$m^3$) at all feedlots. Analytes of interest detected most frequently in downwind samples were moxidectin (81.5%; mean = 0.08 ±0.02 ng/$m^3$), fenvalerate (74.1%; mean = 5.9 ±0.02 ng/$m^3$), permethrin (66.7%; mean = 1.1 ±0.02 ng/$m^3$), and ivermectin (66.7%; mean = 0.32 ±0.1 ng/$m^3$). Relative to upwind samples, doramectin and moxidectin concentrations were significantly higher up to D1 (p <0.001), permethrin and fenvalerate were significantly higher up to D2 (permethrin, p = 0.041; fenvalerate, p <0.001), and ivermectin was significantly higher up to D3 (p = 0.006). While coarse PM size fractions (aerodynamic diameter; <10 - >2.5 μm) may settle out of suspension rapidly, fine PM fractions (0.1 - 2.5 μm) are likely transported beyond the spatial extent of the current study. Moreover, results of the current study significantly expand the known distribution of feedlot-derived pesticides and pharmaceuticals and consequently expand areas of potential ecological impact.

## Track 5: Environmental Risk Assessment

### 5.01 Approaches for Incorporating Complex Data Streams into Risk Assessments

**5.01.T-01 Introductory Remarks: Approaches for Incorporating Complex Data Streams into Risk Assessments**

***Sara M. Vliet**[1] and Risa Sayre[2], (1) U.S. Environmental Protection Agency, (2) University of North Carolina at Chapel Hill*

The session chairs will introduce the session soliciting questions from the audience on strategies for extraction of existing data, integration of heterogenous data and incorporating complex data into decision making paradigms.

**5.01.T-02 Advancing Endangered Species Act Consultations - Use of an Automated, Computational Pipeline to Extract Points of Departure from Public Data Sources**

***Monique Hazemi****, Daniel L. Villeneuve, Andrea LaTier, Mark Jankowski, Carlie LaLone, Christopher M. Schaupp, Manli Chan and Derik Haggard, U.S. Environmental Protection Agency*
Endangered Species Act (ESA) consultations are required when a proposed federal action overlaps a listed species range or designated critical habitat. As part of its ESA consultations, the U.S. Environmental Protection Agency develops species-specific toxicity analyses for each chemical included in the proposed action. Due to substantial workloads, tight regulatory timelines, and the often-protracted length of ESA consultations, there is a need to streamline the development of biological evaluation (BE) toxicity assessments for determining the impact of chemical pollutants on ESA-listed species. Moreover, there is limited availability of species-specific toxicity data for many contaminants, further complicating the consultation process. The current study tested the hypothesis that an automated computational pipeline (i.e., QlikSense application or "app") that extracts, categorizes, scores, and filters data from the ECOTOX knowledgebase would yield toxicity benchmark values that are protective (i.e., lower) compared to those identified through manual search and curation. A combination of 4 species (vernal pool fairy shrimp [*Branchinecta lynchi*], steelhead *[Oncorhynchus mykiss*]*,* Foskett speckled dace [*Rhinichthys osculus ssp.*], and bocaccio rockfish [*Sebastes paucispinis*]) and 12 pesticides, for which BEs have been conducted and toxicity reference values (TRVs) derived, were queried in the app. Within the app, records were scored based on pre-defined data quality characteristics (e.g., taxonomic relatedness to query species). Points of departure (PODs), selected using three different analyses, were collected for each chemical-species pair, and compared to manually curated TRVs. Results indicated that app-derived POD estimates tended to be at lower or approximately equal concentrations relative to manually derived reference values, providing evidence that the app method for automated extraction, filtering, and scoring of effect values reported in ECOTOX yielded generally protective PODs for the evaluated ESA-listed species. However, given that app-derived PODs were not always lower than TRVs, future work may include application of adjustment factors commonly used in BEs. Applying automated data extraction and filtering tools can help standardize the screening of chemicals for evaluation and expedite assessment of available toxicity data to prioritize and select PODs for BE development. *This abstract neither constitutes nor necessarily reflects USEPA policy.*

**5.01.T-03 The ECOTOXicology Knowledgebase Literature Search, Identification and Extraction Protocols: Evolving for Efficiency and Interoperability**

***Dale J. Hoff***[1]*, Jennifer Olker*[1]*, Jason Berninger*[1]*, Katie Nehiba*[2]*, Traci Scott*[2]*, Travis Karschnik*[2] *and Anne Pilli*[2]*, (1) U.S. Environmental Protection Agency, (2) General Dynamics Information Technology*
The ECOTOXicology Knowledgebase (ECOTOX) is a widely recognized source of verified curated single-chemical toxicity data for aquatic and terrestrial organisms. Originally developed by the US Environmental Protection Agency (USEPA) to provide regulators with rapid access to ecological toxicity data, ECOTOX has been providing curated data for use in broad applications in environmental research and risk assessments for over 30 years. ECOTOX continues to evolve to support the need for more information at a faster pace to make regulatory decisions through incorporation of transparent state-of-the-art practices in literature data curation and increased interoperability to other relevant digital applications. The well-established ECOTOX literature review and data curation protocols have now been aligned with current systematic review practices and lexicon for comprehensive literature searches, title/abstract screening followed by full-text review, inclusion/exclusion criteria, and data extraction from acceptable studies following controlled vocabularies. Extracted data contains sufficient chemical, methodological, and toxicity information to support study quality evaluations and data syntheses/reviews by groups internal and external to the USEPA. ECOTOX protocols have recently incorporated automated and semi-automated data analytic tools and techniques including applications for automated literature searching, machine learning and artificial intelligence techniques for title and abstract screening of references, and pre-populating data evaluation records. In addition, ECOTOX has incorporated standard species and chemical identifiers and there are on-going efforts to harmonize terminologies with existing ontologies, which will allow integration with chemical, species, and biological data sources to inform risk assessment. ECOTOX will continue to provide empirical toxicity data, and the recent incorporation of more efficient processes, along with enhanced ECOTOX interoperability with other data sources, will aid in meeting

the current demand for rapid access to toxicity data for risk assessment and environmental research. *This abstract does not necessarily reflect US EPA policy.*

**5.01.T-04 Improving prediction of hydrocarbon biodegradation in aquatic, soil, & sediment systems using system parameterization & machine learning**

*Craig Warren Davis[1], Louise Camenzuli[1], **Aaron Redman**[1], David M Brown[2], Christopher Hughes[2], Delina Lyon[3] and Alberto Martin-Aparicio[3], (1) ExxonMobil Biomedical Sciences, Inc., (2) Ricardo Energy & Environment, United Kingdom, (3) CONCAWE, Belgium*

Evaluation of chemical degradation processes, particularly, biodegradation, is a key element of chemical regulatory management around the globe. Technical complexity and costs associated with environmental biodegradation testing, particularly for UVCB substances, necessitates the advancement of non-testing methods (e.g., quantitative structure-property relationships (QSPRs)). A critical limitation of current models is the inability to incorporate test system and environmental conditions into predictions, leading to uncertainties in model relevance and reliability. This work highlights a novel model (HC-BioSIM) for predicting primary degradation rates (DT50s) of hydrocarbons integrating chemical structure as well as system-dependent parameters, using an expanded database of high-quality petroleum hydrocarbon (HC) DT50 data in water, soil, and sediment systems (N = 728, 1033, & 838, respectively). This modeling framework use system-specific and environmental effects on the biodegradation of petroleum HCs can be quantitatively evaluated in water, sediment, & soil systems. The model reduces uncertainty and provides a better basis for assessment of environmental degradation for hydrocarbon substances. Model performance is greatly improved over previous model frameworks, and no significant bias as a function of HC class, carbon number, or test system parameters were observed. Finally, extensive cross validations demonstrated low variability in model performance as well as parameter usage/importance. This strongly supports a high degree of generalizability of the model for application to external data. The curation of high quality sediment & soil databases improves understanding of the relative persistence properties of hydrocarbons between environmental media, including assessment of the intermedia extrapolation factors used in persistence assessments and exposure models. The model can be applied broadly across regulatory frameworks for risk management.

**5.01.T-05 The multi-sensor data system (MSDS): The strategy and execution of a user-friendly system that puts water quality data, from a variety of different manufacturers, in the hands of scientists**

***Alex Antonison** and Ryan R. Otter, Middle Tennessee State University*

Water quality data is vital to assess both risk and remedy effectiveness in or near water bodies. Although individual manufacturers of water quality devices typically have soft that allows for data downloading and visualizations, the integration of data from different sensors is left to the technical scientist(s). To address this problem, we created the Multi-Sensor Data System (MSDS). This system is designed to automate the ingestion of varying file types into a data lake, process that data into warehouses and marts, and serve up the integrated results via interactive dashboards. Included in this system is the ability for user-defined quality assurance filters, with update in real-time. The processing time need from raw data file upload to dashboard availability is approximately two minutes. This system was built using Amazon Web Services serverless technologies with a focus on low cost and maintenance, while maintaining fast response times and high reliability.

**5.01.T-06 Integration of Regional Data and Modeling for Estrogenic Activity Risk Assessment in the Chesapeake Bay Watershed**

*Stephanie E. Gordon, **Daniel K. Jones** and Brianna Williams, U.S. Geological Survey*

Endocrine-disrupting compounds (EDCs), specifically estrogenic endocrine-disrupting compounds in surface waters may lead to adverse effects in aquatic species at both individual and population levels, often observed through the presence of intersex and vitellogenin induction in male fish. In the Chesapeake Bay Watershed (CBW), located on the mid-Atlantic coast of the USA, intersex has been observed in several sub-watersheds where previous studies have identified specific landscape sources of EDCs in tandem with observed fish health effects. A largescale multi-year effort was conducted to further understand the relationships between landscape sources and drivers and biological effects in smallmouth and largemouth bass, as well as assess the risk of estrogenic activity in surface waters throughout the CBW. This work included the generation of large geospatial datasets using a standardized watershed framework (NHDPlus Version 2.1) to characterize the landscape and identify known and potential sources of EDCs. These datasets were integrated with field data on fish health and water quality to develop interpretive (Classification and Regression Trees) and predictive (Random Forest) models, which were ultimately used to categorize estrogenic activity risk in proximity to management interests (healthy watersheds, public fishing sites). The workflow and results of this effort highlight a system for data identification, summarization, standardization, and integration for machine learning and risk assessments.

**5.01.T-07 Risk Characterization Questions & Discussion**
***Sara M. Vliet***[1] *and Risa Sayre*[2]*, (1) U.S. Environmental Protection Agency, (2) University of North Carolina at Chapel Hill*
This discussion will focus on the current state of the science and the identification of untapped opportunities and needs for future advancement. During this time, questions can be posed to the larger audience or any of the speakers. Discussion topics may include: How do you decide what data is appropriate for a task? How do you figure out from where it should be acquired?; What are the key challenges in using data integrated from many sources in machine learning strategies or computational approaches?; What are some current gaps and challenges in communicating complex data, and modeling, to decision-makers? How can the field move forward in addressing these?; What types of data standards should be in place to ensure maximum data interoperability?

**5.01.T-08 Panel Discussion: Approaches for Incorporating Complex Data Streams into Risk Assessments**
***Sara M Vliet***[1] *and Risa Sayre*[2]*, (1) U.S. Environmental Protection Agency, (2) University of North Carolina at Chapel Hill*
This panel discussion will follow on the risk characterization questions posed and discussion in the previous time slot.

**5.01.P Approaches for Incorporating Complex Data Streams into Risk Assessments**

**5.01.P-We159 The Pesticide Indirect Photodegradation (PIP) database: A new data-sharing platform for screening of existing and development of new agrochemicals**
***Zachary Stickelman****, Natalie Clay, Jessica Huang, Jessica Lewer, John Peloquin and Jakub Kostal, George Washington University*
The design of safer chemicals and the design for degradation are among the key principles of Green Chemistry. These principles extend to pesticides, which are a class of specialty commercial chemicals with intended biological function, i.e. selective toxicity. To that end, designing pesticides with controlled depletion and low (unintended) ecotoxicity, while maintaining good efficacy, is one of the greatest challenges of industrial green chemistry. We have recently developed and validated several computational approaches to aid in the development of safer agrochemicals, mimicking the successful blueprint of computational drug discovery. To help disseminate our findings to industry and regulators, here we present a data-sharing platform that integrates available experimental and our own predicted data for over 700 agrochemicals on the US EPA's registry. At the core of our platform is a series of *in silico* models, which link structural and substructural attributes of pesticides to process metrics related to indirect photodegradation, ecotoxicity, and intended function. The database is searchable by structural and non-structural identifiers, where outputs provided to the end-user include our assessment of underlying data uncertainty and variability to aid in practical decision-making. We envision this platform can serve as a useful guide for hazard and alternatives assessments, where distribution metrics along with pesticides structurally similar and relevant to the user query are provided as output. Lastly, the platform can be employed as a tool for the redesign of existing and discovery of new agricultural products

by levering incorporated designed vectors and target-specific information. The overarching goal of this project is to spur grander efforts in toxicological data-sharing, which would enable more effective dialog about chemical performance and safety and the utilization of NAMs (New Approach Methodologies) across academia, industry, and government.

**5.01.P-We160 Considerations for the Use of Estimated and Measured Environmental Media Concentrations to Derive Environmental Risk in TSCA Risk Evaluations**

***Sarah Au***, *Chantel Nicolas, Kara Koehrn and Jim Bressette, U.S. Environmental Protection Agency*

Under the Frank R. Lautenberg Chemical Safety for the 21st Century Act, which amended the Toxic Substances Control Act (TSCA), the U.S. Environmental Protection Agency (EPA) is required to conduct risk evaluations on chemical substances by evaluating both hazard and exposure, without consideration of costs or other non-risk factors, using the best available science and weight of the scientific evidence. Under TSCA, human and environmental risks are evaluated in the context of the condition of uses (COU) associated with the chemical undergoing risk evaluation. Thus, exposure data used to evaluate each COU may differ based on the availability and relevancy of information such as facility-specific releases and measured monitoring data. Furthermore, the availability of facility- or COU-specific data dictates the estimation approaches (modeling) for evaluating abiotic (e.g., surface water, sediment, air) and biotic (e.g., tissue residues) environmental media concentrations that may be used if the domains of applicability are relevant for the respective environmental media and exposure pathway. Models such as the Exposure and Fate Assessment Screening Tool (E-FAST), Point Source Calculator (PSC), and $K_{ow}$ (based) Aquatic BioAccumulation Model (KABAM) have been used to estimate environmental media concentrations in previously conducted risk evaluations under TSCA. Another consideration that determines which exposure data are used to evaluate environmental risk associated with a specific COU, is whether there exists relevant and applicable hazard data. Two of the first ten chemicals to undergo risk evaluations under TSCA, hexabromocyclododecane (HBCD) and trichloroethylene (TCE), used different approaches for evaluating environmental exposure resulting from COU-specific industrial releases. Environmental exposure and hazard data landscapes as well as the chemical- and COU-specific considerations used to characterize environmental risk for these two chemicals, are compared to illustrate the breadth of information sources and their accompanying uncertainties that resulted in their associated risk estimates. *The views expressed in this presentation are solely those of the authors and do not represent the policies of the U.S. EPA. Mention of trade names or commercial products should not be interpreted as an endorsement by the EPA.*

**5.01.P-We161 Prioritizing Chemicals of Emerging Concern in the Great Lakes Using Covariance of Chemical Concentrations and Diverse Biological Responses**

***Kelsey Vitense*[1]**, *Brett R. Blackwell[1], Timothy W. Collette[1], Steven Corsi[2], Christine M. Custer[2], Erik Davenport[3], Drew R. Ekman[1], Steph Hummel[4], Satomi Kohno[5], Luke Loken[2], Erin Maloney[6] and Edward Perkins[7], (1) U.S. Environmental Protection Agency, (2) U.S. Geological Survey, (3) National Oceanic and Atmospheric Administration (NOAA), (4) U.S. Fish and Wildlife Service, (5) St. Cloud State University, (6) University of Minnesota, (7) U.S. Army Engineer Research and Development Center*

The Great Lakes Restoration Initiative (GLRI) aims to protect and restore the nation's largest freshwater resource, including consideration of the adverse impacts of complex chemical mixtures and contaminants of emerging concern (CECs) on Great Lakes fish and wildlife. To address this issue, a multi-agency research consortium conducted field studies at sites along the Maumee River in Ohio, USA in 2016-2017, monitoring >700 organic compounds along with *in vitro* and *in vivo* biological effects (e.g., transcription factors, gene expression, metabolomics, body weight, embryo deformities and time to hatch) in ecologically relevant species (fathead minnows, tree swallows, and Asian clams). The goals of the present study are to: 1) identify chemicals that consistently covary with biological effects across studies and species to help prioritize chemicals for future monitoring and management efforts, and 2) identify chemicals that consistently display a lack of covariance across studies and species to help “shorten the list” of contaminants that require monitoring. To meet these goals, we employed sparse partial least squares regression to identify chemicals with statistically significant relationships within each species-response group of interest, and quantified strength of evidence for each chemical using selection frequency in models exhibiting the best predictive performance. We synthesized these results to quantify the strength of evidence of biological impacts across all studies and species responses. Finally, we incorporated chemical detection frequency and the number of species-response groups analyzed to develop a weight-of-evidence approach to prioritize chemicals based on relationships with biological effects, relative occurrence in the environment, and relative availability of biological information. The results from this study will help identify high and low priority chemicals for future monitoring and natural resource management in the Great Lakes.

**5.01.P-We162 Standardized Application of NAMs in Chemical Risk Assessment**

***Kelly Magurany**, NSF*

Incorporation of diverse data streams to inform mode of action and strengthen the weight of evidence for risk decision-making is best practice in risk assessment. Effective application of data developed through new approach methodologies (NAMs) requires careful evaluation of chemical toxicokinetics and integrity and interpretation of the methods and results. Further, consideration of uncertainty in quantifying estimated *in vivo* exposures from *in vitro* assays is critical in deriving comparative points of departure. To standardize the approach, a framework that outlines the thought process for applying NAMs in risk assessment was developed. This framework includes the available data sources, methodologies and interpretive resources to effectively apply NAMs. In addition, tools and methods for quantitative extrapolation to *in vivo* conditions and associated uncertainties are discussed. With this holistic view of the NAMs space, utilization of these data in risk assessment may be standardized and more easily understood and accessible.

**5.01.P-We163 Screening and Prioritization of Contaminants of Emerging Concern and Mixtures in the Puget Sound**

***Maya Faber**[1], Christopher Andrew James[2] and Ruth M. Sofield[1], (1) Western Washington University, (2) University of Washington*

A variety of anthropogenic chemicals known as contaminants of emerging concern (CECs), are regularly released into the aquatic environment due to human activities. CECs include substances used in daily human life, such as antibiotics, personal hygiene products, and pharmaceuticals, as well as chemicals used in manufacturing, as flame retardants, pesticides, and thousands of other consumer products. These substances are identified as CECs because their environmental occurrence and toxicology are poorly characterized. Considering the number and diverse range of CECs, it is important to screen and prioritize chemicals and chemical mixtures that pose the greatest threat to environmental health. This work applies a risk characterization approach to screen and prioritize CECs in the Puget Sound (United States) based on their potential to cause biological impacts. Regional monitoring data was compiled across several sampling campaigns, which provide information on CEC concentrations in surface water, wastewater, mussel tissue, and fish tissue. To overcome the gap in traditional ecotoxicity data for CECs, measured environmental concentrations were compared to existing biological effects concentrations reported *in vivo,* using the NORMAN ecotoxicology database, and *in vitro,* from the ToxCast database. A biological response ratio (BRR) was calculated by dividing the measured concentration by the biological effects concentration to classify priority chemicals by potential to result in biological impacts. Priority chemicals are those with a greater likelihood to cause a biological response, and have a higher BRR. Mixtures effects are estimated by identifying chemicals with common endpoints, gene targets, or Adverse Outcome Pathways, which may result in the same biological response. Therefore, in addition to evaluating chemicals individually, chemicals are also identified which may have a low BRR but when considered as a mixture may be acting additively, increasing the biological effect. As a result of this study, CECs are categorized into three groups, high priority chemicals with a likely biological effect, watch list chemicals with a potential for a biological effect, and those chemicals with low potential for biological effects. CEC screening and prioritization can then be used to direct management and regulatory efforts to priority compounds.

## 5.02 Bayesian Networks in Environmental Risk Assessment and Management

### 5.02.T-01 1. To Explore Strange New Worlds, the future of Bayesian networks, Risk Assessment and Adaptive Management

***Wayne G. Landis****, Western Washington University*

The application of Bayesian networks (BN) and other tools have proven useful in the estimation of risk to contaminated sites, oil spills, the effects of climate change to coral reefs, the estimation of quantitative structure activity relationships, and to measure the resilience of an urban area to sea level rise. The structure of a BN, its ability to represent multiple cause-effect pathways, to incorporate population models in concert with molecular effects pathways, and the determination of the utility of management options are powerful tools in applying an adaptive management strategy. There are new areas of investigation. Ecological risk assessments for micro and nanoplastics are just now being completed. Synthetic biology, especially the application of gene drives to control insect that carry human disease or to control invasive species is a new area for ecological risk assessment and BNs have proven applicable to these assessments. The effects of climate change in concert with the classical contaminant driven scenarios such as CERCLA sites can be addressed using these approaches. Perhaps more important is the application of the overall process to a reconsideration of how risk assessment can be a framework by which to plan future investigations and to focus on the characteristics of a new microplastic or pesticide that are most critical in making a robust ecological risk assessment with experiments that describe exposure-response, exposure within landscapes applicable to decision making and to create long-term data sets that are suitable to machine learning and other tools. It appears to be possible to connect both ecological and human health and well being into an overall risk assessment process. Furthermore, this process can be innately applied to the long-term adaptive management of environmental systems integrated with appropriate societal measures to provide long-term and reliable ecosystem services.

### 5.02.T-02 A Probabilistic Ecological Community Analysis for Coral Reef Systems in Puerto Rico

***John F. Carriger*** *and William Fisher, U.S. Environmental Protection Agency*

Effects analysis in ecological risk assessment requires choosing endpoints that are representative of ecological communities. Indicators of different components of the community and summary indicators can both be chosen. However, summary indicators applied to a complex system can sometimes result in metrics that are ambiguous due to mathematical difficulties and loss of interpretability. Coral reefs are complex ecosystems, so patterns of ecological interactions were explored by probabilistic clustering of reef monitoring variables with Bayesian networks. This could provide objective insights that would be undetected from informal and subjective indicator approaches. In 2010 and 2011, scientists from the U.S. Environmental Protection Agency sampled coral reef communities along the coast of Puerto Rico with probabilistic surveys. These data were used in a multivariate analysis with Bayesian networks. Relationship analysis approaches such as a maximum spanning tree Bayesian network structures were used to detect and characterize correlations. Most of the sub-community variables such as gorgonians, sponges, fish, and coral variables were found to have stronger associations within than between sub-communities. Complexity weights for the score-based learning algorithm were lowered to ensure a fully connected network that contained all variables. A second phase used a variable clustering analysis to identify clusters for sponge, gorgonian, stony coral and fish monitored variables. These clusters were constructed using an expectation-maximization algorithm that created a factor node for each taxon with clusters identified by the algorithm. The clusters were interpreted in terms of their relationship with the monitoring variables used in their construction and the relationship to the monitoring variables for other taxa, such as stony coral clusters with fish variables. Each of these factor nodes were then used to create a meta-factor variable that further summarized the entirety of the coral reef community monitoring variables. Further work can be used to examine the clusters identified on a regional or site-specific basis and interpret the cluster characteristics in terms of known sources and levels of stressors to support ecological risk assessment and coral reef protection decisions. EPA disclaimer: The views expressed in this presentation are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

**5.02.T-03 An Ecological Risk Assessment for Microplastics in the San Francisco Bay Using the Bayesian Network Relative Risk Model**

***Emma Sharpe***, *Wayne G. Landis, Skyler Elmstrom and Erika Whitney, Western Washington University*

There has been an increased interest in understanding and managing the impacts that microplastics may have on ecological systems because recent studies have shown that plastic particles are widespread in the environment and that exposure to these particles has toxicological effects. Until now, an ecological risk assessment for microplastics that meets the current standards for risk assessment, has not been completed. Here we present an ecological risk assessment for a specific type of microplastic in the San Francisco Bay. This study lays the groundwork for future ecological risk assessments of microplastics and identifies key uncertainties that need to be addressed. Using a Bayesian network relative risk model (BN-RRM), we determined the risk tire wear particles present to juvenile Chinook salmon and Northern anchovy. In past studies, BN-RRM has been a successful framework for regional scale ecological risk assessments of multi-stressor systems, allowing for the creation of a model with predictive capability and adaptive potential as new data become available. The BN-RMM is parameterized for each risk region with tire wear particle environmental concentration data collected by the San Francisco Estuary Institute, plastic particle toxicity data generated by Oregon State University, and site-specific water quality, chemical, and land use data from regional databases. Relative risk was then calculated for each risk regions and spatial gradients of risk were determined. Results indicate a relatively low risk for juvenile Chinook salmon and Northern anchovy at current tire wear particle concentrations in the San Francisco Bay. This risk assessments confirms that, with the data that is currently available, a quantitative, spatially specific risk assessment is possible. Additionally, Bayesian networks are an excellent tool for modeling the complex and uncertain nature of microplastics. This study is funded by the National Science Foundation Growing Convergence Research Grant (1935018) program.

**5.02.T-04 Prediction of pesticide effects on aquatic communities in a rice field by using a Bayesian network approach - A southern European case study**

***Sophie Mentzel***[1], *Claudia Claudia Martínez*[2], *Andreu Rico*[2], *Merete Grung*[1], *Knut Erik Tollefsen*[1], *Paul van den Brink*[3] *and Jannicke Moe*[1], *(1) Norwegian Institute for Water Research (NIVA), Norway, (2) IMDEA Water Institute, Spain, (3) Wageningen University & Research, Netherlands*

In recent years, Bayesian networks (BN) have become a more popular tool to support probabilistic environmental risk assessments. They can better account for uncertainty compared to the deterministic approaches currently used in traditional risk assessment. In this study, we aim to estimate the potential effects of various pesticides on the aquatic ecosystem of rice fields surrounding a Spanish Natural Park. We linked inputs and outputs of a process-based exposure model (RICEWQ) together with probabilistic case-based effect model (PERPEST) by used the BN as a meta-model. The exposure prediction model was run for various meteorological, hydrological and pesticide emission scenarios. The final developed BN enables the prediction of pesticide effects on several response classes of the taxonomic and functional groups in an aquatic ecosystem. This approach also facilitates the communication of uncertainties associated with the effect assessment in a transparent way.

**5.02.T-05 Bayesian Network Human Health Risk Assessment of Per- and Poly-Fluorinated Substances (PFAS) via Fish Consumption at Multiple Waterbodies in Texas**

***Eric Lawrence***, *Heidi K. Bojes, Andrew Myers, Ketki Patel and Kirk Wiles, Texas Department of State Health Services*

DSHS is currently strengthening environmental health capacity to detect, prevent and control environmental health hazards through data-driven, evidence based approaches. As part of these efforts, DSHS is analyzing per- and poly-fluorinated substances (PFAS) concentrations in fish species in multiple waterbodies in Texas and assessing the associated risk to human health via fish consumption. DSHS staff sampled a total of 60 total fish from five species at 10 locations in Lake Worth, Texas in 2020 and sampled 52 total fish from seven species in three locations in Lower Leon Creek, Texas in 2021, along with water and sediment samples at each sampling location. We analyzed fish tissues for 28 PFAS compounds and are using a Bayesian network as part of our risk assessment. This presentation will outline our fish sampling and data analysis methods, including the use of Bayesian network, a probabilistic statistical model, to quantify health risks associated with consumption of PFAS contaminated fish.

**5.02.T-06 Identifying and testing adaptive management options to increase river catchment resilience using a Bayesian Network.**

***Kerr Adams***[1]*, Christopher (Kit) A.J. Macleod*[2]*, Marc J. Metzger*[1]*, Nicola Melville*[3]*, Rachel C. Helliwell*[2]*, Jim Pritchard*[3]*, Katie Edwards*[4] *and Miriam Glendell*[2]*, (1) University of Edinburgh, United Kingdom, (2) The James Hutton Institute, United Kingdom, (3) Scottish Environment Protection Agency, United Kingdom, (4) Scottish Water, United Kingdom*

The cumulative impacts of future climatic and socio-economic change threaten the ability of freshwater catchments to provide valuable socio-ecological services. Stakeholders who manage freshwater resources require decision-support tools that increase their understanding of catchment system resilience and support the appraisal of adaptive management options. Our research aims to address the following question: Can a Bayesian Network (BN) model support stakeholders in the identification and testing of adaptive management options that help increase catchment system resilience to the impacts of cumulative future change? Using the predominantly arable Eden catchment (320km$^2$), in eastern Scotland as a case study, we applied participatory methods to develop a BN model to simulate the resilience of the catchment to the impacts of future change.

The BN model coupled climatic and socio-economic factors to a 2050 time horizon. We applied continuous nodes within the hybrid equation-based model to measure the uncertain impacts of both climatic and socio-economic change, which was a novel way of considering 'what if?' scenarios within a BN model. Model outputs provided stakeholders with a holistic systems-thinking approach for measuring catchment scale resilience to the cumulative impacts of future change.

Catchment resilience outputs informed the identification of adaptive management options during stakeholder workshops. Adaptive management options were grouped into management scenarios and their effectiveness in increasing catchment system resilience was tested using the BN model. Two optimal adaptive management scenarios were identified; the first optimal management scenario focused on predominantly nature-based management options such as wetland wastewater treatment methods and rural sustainable drainage systems. The second optimal scenario focused on resource recovery, including phosphorus recovery from wastewater treatment works and constructed lagoons for crop irrigation.

Our BN model application supported the identification and testing of adaptive management options, providing stakeholders with a strategic systems-thinking approach for considering river catchment resilience. Our participatory methods facilitated collaboration and innovation within the management scenarios identified. The ability of the BN model to test and compare adaptive management scenarios in a time-effective manner was seen as an advantage in comparison to conventional methods.

**5.02.T-08 Lessons Learned and Recommendations for Using Bayesian Networks in Environmental Risk Assessments**

*Mariana Cains*[1] *and* ***Wayne G. Landis***[2]*, (1) National Center for Atmospheric Research, (2) Western Washington University*

This presentation offers a summary of and lessons learned from the diverse applications of Bayesian networks within the field of environmental risk assessment. This summary highlights the utility of Bayesian network analysis as a flexible decision support approach that 1) integrates qualitative data (e.g., expert and community knowledge, governing regulations) and quantitative data (e.g., chemical toxicity, climate projections), 2) inherently accounts for data uncertainty (e.g., missing data) and variability (e.g., parameter distribution), and 3) generates counterfactual scenarios that can be use to evaluate potential management options (e.g., remediation, mitigation). The utility of Bayesian networks is further emphasized by the ease in which the underlying causal logic is understood by researchers, decision makers, and stakeholders a like. In order for researchers and decisions makers to reap the benefits of Bayesian network analysis, assessment and management approaches must 1) employ stakeholder-based and stakeholder-participatory research methods that acknowledge and treat the stakeholders members as collaborators, rather than just subjects for data collection, 2) integrate region and research question relevant data from disparate disciplines and data sources, and 3) evaluate stakeholder-centered solutions through the measurement of qualitative and quantitative management metrics that are assessed through the adaptive management lens. Bayesian network analysis is not a "silver bullet" that will serve up the perfect management solution. Rather, Bayesian network analysis offers a systematic approach to evaluate convergent and wicked management challenges that require input from multiple sources of knowledge to find feasible management options.

## 5.02.P Bayesian Networks in Environmental Risk Assessment and Management

### 5.02.P-Tu132 Characterizing Assets, Threats and Solvability with Bayesian Networks to Support Spatial Prioritization for Environmental Management

***John F. Carriger*** *and Susan Yee, U.S. Environmental Protection Agency*

A screening-level spatial prioritization framework is proposed using Bayesian networks and Stefan Hajkowicz and colleagues' Assets, Threats, and Solvability (ATS) framework. The ATS framework is based on multicriteria decision analysis and provides a useful way for organizing information needed in prioritization assessments. In ecological risk assessment, prioritizing spatial regions for protection often includes a consideration of the components of the ATS framework- the assets are often related to valued ecological attributes, the threats are often from the chemical hazards, and solvability is related to the degree of effectiveness for restoring or protecting a location. The calculations necessary to implement ATS are possible with or without a Bayesian network. However, the Bayesian network can also allow incorporation of uncertainties for each of the component attributes. A schematic for using the ATS with Bayesian networks follows the decision analysis framework and starts with a selection of a decision context. Next, the ATS criteria are developed using an objectives hierarchy. An objectives hierarchy is especially useful for defining the criteria for a decision and for ensuring the criteria are internally cohesive and robust for analyzing trade-offs. A Bayesian network model is then developed to calculate the ATS hierarchy and compute prioritization levels as well as levels of the individual components of the ATS. Depending on the context, the ATS Bayesian network can be used for a variety of spatial scales and types from a large region to a grid cell. The ATS framework with Bayesian networks is potentially useful for summarizing complex information needed for protection, conservation, and restoration decision contexts where spatial differentiation and prioritization are key components of the environmental assessment. EPA Disclaimer:The views expressed in this presentation are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

### 5.02.P-Tu133 Bayesian Network Model of mercury risk to the environment and stakeholders in the Mackenzie River Basin

***Una Jermilova***[1]*, Holger Hintelmann*[1]*, Jannicke Moe*[2]*, Wayne G. Landis*[3] *and Jane Kirk*[4]*, (1) Trent University, Canada, (2)Norwegian Institute for Water Research (NIVA), Norway, (3) Western Washington University, (5) Environment and Climate Change Canada*

This study is the Canadian contribution to ARCRisk (Risk evaluation, risk reduction and risk management action plans for mercury in the Arctic – a circumpolar management approach), a multilateral collaborative project between Canada and Norway. The project will use Bayesian-Network Relative Risk models (BN-RRMs) to identify targeted risk reduction measures for mercury (Hg) releases from key sources to land and water in the Arctic. The Mackenzie River Basin (MRB) is the Canadian study site. The MRB covers 20% of Canada's landmass and is the fourth largest freshwater contributor to the Arctic Ocean.

Taking into account the geological diversity of the MRB, and the spatial distribution of numerous mercury sources, we intend to use an integrated Bayesian - Network Relative Risk model (BN-RRM) model capable of analyzing a multi-stressor, multi-endpoint system. BN-RRM is a deterministic model that uses probability distributions to estimate risk, and by nature will incorporate uncertainty. A BN-RRM can integrate observations from various sources including environmental models and expert opinion into a single framework. They are visual representations of cause-and-effect relationships that can facilitate communication of complex interactions to stakeholders.

For the MRB, risk is determined for four endpoints: 1) the health and cultural impact to the Northwest Territories Indigenous Canadians; 2) the risk to health of aquatic and terrestrial organisms of the MRB; 3) the threat to recreational and commercial fisheries; and 4) risk of increased Hg load into the Arctic Ocean. Included is a review of sources and identification of key measures, aimed at recommending concrete reduction measures with high relevance for a larger part of the Arctic. Once developed, the model can use diagnostic analysis methods to predict what variable is driving the risk to these endpoints. When an influential stressor is identified, management nodes can be implemented. The model may be applied to compare management strategies, their predicted influence on the risk to endpoints, and the associated uncertainties.

## 5.03.P Benefits and Obstacles in Using Mechanistic Effect Models for Chemical Risk Assessments

### 5.03.P-Mo146 Realism, Conservatism, and Tiered Ecological Risk Assessment

***Matthew Etterson**, U.S. Environmental Protection Agency*

Recent research has provided valuable momentum for the development and use of population models for ecological risk assessment (ERA). In general, ERA proceeds along a tiered strategy, with conservative assumptions deployed at lower tiers that are relaxed at higher tiers with ever more realistic models. As the tier increases, so do the levels of time and effort required by the assessor. When faced with many stressors, species, and habitats, risk assessors need to find efficiencies. Conservative lower-tier approaches are well established, but higher-tier models of-ten prioritize accuracy, and conservative approaches are relatively unexplored at higher tiers. A principle of efficiency for ecological modeling for population-level ecological risk assessment is articulated and evaluated against a conceptual model and an existing set of avian models for chemical risk assessment. Here, four published avian models are reviewed in increasing order of realism (risk quotient →Markov chain nest productivity model → endogenous lifecycle model → spatially explicit population model). Models are compared in a pairwise fashion according to in-creasing realism and evaluated as to whether conservatism increases or decreases with each step. The principle of efficiency is shown to be a challenging ideal, though some cause for optimism is identified. Strategies are suggested for studying efficiency in tiered ecological model deployment.

### 5.03.P-Mo147 Understanding and Overcoming Factors Influencing the Uptake of New Approach Methodologies by Ecological Risk Assessors

***Austin Wray**, Daniel Aboagye and Thomas M. Steeger, U.S. Environmental Protection Agency*

New approach methodologies (NAMs) largely reliant on high throughput *in vitro* and *in silico* tools are continuing to evolve as a means of replacing, refining, and reducing reliance on more resource intensive *in vivo* assays. However, the willingness of some ecological risk assessors to adopt these tools has been limited by uncertainties regarding the extent to which the NAMs reflect whole organism and/or population/community-level responses across broad taxonomic and chemical domains. This presentation will discuss factors which have limited the uptake of NAMs and how these evolving technologies may gain greater acceptance by risk assessors. The incremental use of NAMs at various stages of the risk assessment process coupled with evidence that that these tools provide predictive/protective (*i.e*., reasonably conservative) measures of exposure and effects within and across taxa may provide the necessary evidence to influence uptake of these methodologies by risk assessors.

**5.03.P-Mo148 Mechanistic effect models: A brief history to highlight benefits and obstacles in using them for chemical risk assessment.**

***Chiara Accolla***[1]*, Valery Forbes, Nika Galic*[2]*, Sandy Raimondo*[3]*, Amelie Schmolke*[1] *and Maxime Vaugeois*[2]*, (1) Waterborne Environmental, Inc., (2) Syngenta Crop Protection, (3) U.S. Environmental Protection Agency*

Although mechanistic effect models are widely recognized as potentially valuable tools, they are still not regularly used or accepted for regulatory Ecological Risk Assessments (ERA). There are several possible reasons for this, including the lack of trust and transparency in the modeling process, but more importantly the mismatch between the endpoints they yield and those that have been traditionally applied and the lack of "bright lines".

This presentation aims to open a thorough discussion among all the parties involved in the risk assessment process to understand how we could enhance the use of mechanistic effect models in different regulatory contexts. To this end, we present a brief history of mechanistic effect models for ERA and give an overview of their goals within the ERA context. We show some examples of effect models developed within different stakeholders (US EPA, academia, business) and highlight their similarities and differences.

In this context, we suggest focusing on a few important points:

- Define standardized outputs of interest, such as population abundance, population decline, recovery, or extinction probability and how these outputs can be applied in decision making.
- Determine which and how environmental scenarios should be applied across models.
- Find agreement on those model features deemed essential to represent populations such that risks can be adequately assessed.
- Underline the importance of identifying model use and objectives before model development and ensure transparency and consistency in the overall process.

We also tackle some common issues linked to model acceptance and conceptual misunderstandings. For example, models are currently often newly developed or adapted for the context of a specific risk assessment and thus, include different processes relevant to the system and objectives. This reduces consistency across models and increases the effort involved in reviewing them. Moreover, there are different points of view regarding how to best use data obtained from surrogate species, how to deal with data gaps, and how to address model uncertainty. In conclusion, we hope to foster dialog among stakeholders to ensure the use of the best available science in a standardized way to support ecological risk assessments of chemicals.

**5.03.P-Mo149 Accounting for temperature-dependent toxicity in mechanistic effect models: the case study of Chironomus riparius exposed to thiamethoxam.**

***Maxime Vaugeois***[1]*, Josef Koch*[2]*, Silke Claßen*[2]*, Daniel Gerth*[2]*, Tido Strauss*[2]*, Jay Overmyer*[1] *and Nika Galic*[1]*, (1) Syngenta Crop Protection, (2) Research Institute gaiac, Germany*

Thiamethoxam (TMX) is a neonicotinoid insecticide that is applied in North America as seed treatment, soil, and foliar uses primarily from April to August. The most recent ecological risk assessment released by the US EPA identified potential risk concerns primarily from chronic TMX exposure to freshwater aquatic invertebrates, with chironomids identified as one of the most sensitive taxa. Chironomids are a diverse group of primary consumers commonly found in high abundance in many freshwater ecosystems. As such, they represent a primary prey item for many species, including species listed under the Endangered Species Act (ESA), indicating that indirect effects on listed species could be a concern. However, given the temporal aspects of potential exposure and chironomid life cycles, a more quantitative assessment of potential effects was needed.

The goal of this study was to quantify effects on individual chironomids from exposure to TMX under realistic environmental conditions. To that end, we conducted laboratory experiments to calibrate and validate temperature-dependent models. We used the General Unified Threshold model of Survival (GUTS) to simulate survival, and an existing Dynamic Energy Budget (DEB) model, which included a Toxicokinetic-toxicodynamic (TKTD) model, to simulate sublethal endpoints, including survival, growth, reproduction, and development of *Chironomus riparius*. The experiments were conducted at multiple TMX concentrations and constant temperatures to quantify the temperature dependency of TMX toxicity. Then, the GUTS and DEB-TKTD models were validated using data generated in tailor-made experiments. In this new set of experiments, chironomids were exposed to pulsed TMX concentrations under either a constant or a dynamic (increasing) temperature regime. Finally, we applied the validated models to evaluate TMX exposure profiles obtained from water monitoring data collected in vulnerable watersheds with intense TMX use under realistic temperatures.

Our results showed that TMX toxicity is temperature dependent. Moreover, we found that exposure to TMX resulted in decreased survival and reduced growth, and very little to no impact on reproductive output, consistent with other studies. Validation of the model showed very good performance for the dynamic temperature regime, thus confirming that the temperature-dependent toxicity of TMX was adequately captured.

**5.03.P-Mo150 A New Software Tool for Promoting Standardization of Conceptual Diagrams for Mechanistic Effect Models**

*Kristin Crouse[1], **Valery Forbes**[1], Chiara Accolla[2], Thomas Banitz[3], Nika Galic[4], Volker Grimm[3], Sandy Raimondo[5], Amelie Schmolke[2] and Maxime Vaugeois[4], (1) University of Minnesota, (2) Waterborne Environmental, Inc., (3) Helmholtz Centre for Environmental Research – UFZ, Germany, (4) Syngenta Crop Protection (5) U.S. Environmental Protection Agency*

Due to lack of guidance, risk assessors and risk managers have shown reluctance to use mechanistic effect models for ecological risk assessment. Recent efforts have promoted guidance in documentation (e.g., ODD, TRACE), evaluation (e.g., TRACE, Pattern-Oriented Modeling) and development (e.g., Pop-GUIDE). However, guidance is still needed for how to build conceptual model diagrams, which visually communicate the salient details of a model to a general audience. Currently, modelers create conceptual model diagrams using a wide variety of approaches, such that two modelers depicting the same model would likely yield vastly different diagrams. To reduce individual bias in diagram construction, we propose a new software tool that produces standardized and consistent diagrams for any kind of mechanistic effect model. Users will visit a public webpage and answer a series of questions about their model. The software will generate a visual diagram from these responses, which the user can download for free. The diagram will include information on key elements of a mechanistic effect model, including: (1) properties of the environment such as spatial heterogeneity, external drivers, or chemical exposure; (2) organism characteristics such as life-history traits, behavior, and energetics; (3) other key features such as density dependence and stochasticity; and (4) important model outputs such as abundance, biomass, and more. In the generated diagram, these elements are both listed as text and depicted visually to show their connections, thus highlighting the main features of the model while being consistent across models. We expect that our standardized diagrams will be quick and simple to understand, capturing the key features of the model without going into too much detail, and be applicable to a wide range of model types and complexities. Ultimately, these improvements will promote transparency in model descriptions and will cultivate trust among modelers, assessors and managers.

**5.03.P-Mo151 The Application of Population Models, Quantitative Adverse Outcomes, and similar tools in Ecological Risk Assessment**

***Wayne G. Landis**, Western Washington University*

For decades the use of Quotient Method as an indicator of risk has existed despite its obvious limitations in depicting probability, the size of the effect, and the associated uncertainties. Population models, toxicokinetics and other tools have long been available to provide a picture of risk that clearly depicts a probability distribution and the uncertainties with the estimate. The more widespread use of Bayesian networks and related techniques have made the inclusion of exposure-response curves, population models, biochemical pathways and other quantitative approaches essentially routine. An often used excuse is that these approaches are too difficult or not required by regulation. Yet my experience and others is that these tools have been used in many case studies and are now a part of the regular literature. So what are the issues? One, perhaps much of the field of environmental toxicology and risk assessment is comprised of those whose focus in on the molecular and organismal effects of toxic materials. Their knowledge of population biology, population genetics and ecology may be limited. Conversely those who have a background primarily in the ecological arena are not comfortable thinking about active sites or quantitative structure activity relationships. As a professor in environmental toxicology and risk assessment for four decades my concern is that we have not taught that both are necessary to evaluate risk. There are now also a variety of quantitative tools now available so that climate change, molecular effects, population dynamics and landscape interactions can all be incorporated into a risk assessment. It will also be necessary to start bringing regulation and practice to the state of the art for the early 2020s. The presentation will provide several case studies of how this is done.

**5.03.P-Mo152 Scenario Development for Bee Risk Assessment and Health Modelling**

***Christopher M. Holmes*[1]*, Thorsten Schad*[2]*, Sascha Bub*[3] *and Thomas Preuss*[2]*, (1) Applied Analysis Solutions LLC, (2) Bayer CropScience LLC, (3) University of Koblenz and Landau, Germany*

Bee effect modelling has become a core instrument in bee risk assessment in Europe (EFSA 2013 '*Guidance Document on the risk assessment (RA) of plant protection products (PPP) on bees'*, EFSA 2018 '*A systems-based approach to the environmental RA of multiple stressors in honey bees'*, EFSA 2021 *'Outline of the revision of the Guidance on the RA of PPPs on bees*'). Pesticide RA using such models is based on scenarios. In this project we (i) identified key conceptual elements of scenarios in regulatory RA, (ii) developed proposals for these elements, and (iii) generated scenarios for the BEEHAVE model to demonstrate these elements. The example assumed a RA for honeybees related to the use of a PPP in apples located in France. As apple cultivation density increases so does attractiveness of sites for honeybee keeping (occurrence) and exposure potential. Therefore scenario representativeness (level of conservatism) was mainly driven by regional apple cultivation density. Three regions were selected located in different climatic regions to account for weather variability. In these regions, local site selection (i.e., placing the beehive) was done in a combination of bee forage mapping at medium resolution and beekeeper preference. For each site, scenarios were constructed for a 9 km radius around the beehive. In view of a scenario development framework, we propose a tiered scenario development scheme to implement a well-defined level of complexity together with a targeted certainty evaluation. We developed structured bee forage information layers for transparent bee forage modelling: (i) Land use/cover, (ii) Vegetation, (iii) BeeForage. All information layers are spatially and temporally explicit. The transition from one information layer to the next was done by explicit modules, e.g., the BeeForage module represents an approach on how nectar and pollen provision is modelled for a given vegetation and its phenology. According to the tiered scenario scheme, simple modules can be replaced with more sophisticated ones if needed and available. In our example we start with simple representations of processes, e.g., bee forage (nectar and pollen) provision is modelled in five categories (0-4, 4=mass forage) represented in a lookup table defined from literature, by vegetation patches with a monthly resolution vegetation phenology. Due to current technical limitations of BEEHAVE, the spatiotemporally explicit BeeForage(x,t) information is aggregated into <500 units.

**5.03.V Benefits and Obstacles in Using Mechanistic Effect Models for Chemical Risk Assessments**

**5.03.V-01 An overview of modelling approaches for ecological risk assessment of pesticides**

***Sandrine Charles***[1]*, Floriane Larras*[2]*, Arnaud Chaumot*[2]*, Celine Pelosi*[2]*, Laure Mamy*[2,3] *and Rémy Beaudouin*[4]*, (1) University Lyon, France, (2) INRAE (Institut National de la Recherche Agronomique), France, (3) Institut national de la recherche scientifique (INRS), Canada, (4) Institut National de l'Environnement Industriel et des Risques (INERIS), France*

A wide diversity of pesticides are used for crop protection leading to the contamination of soil, water and air, and can therefore have ecotoxicological impacts on living organisms. It is inconceivable to study effects of each compound on each model species from each ecosystem compartments, experimental studies being time consuming and cost prohibitive, and animal testing having to be avoided. Therefore, numerous models are developed to assess ecotoxicological effects of pesticides. In this context, our objective was to review modelling approaches enabling the assessment of the effects of pesticides, including bio-control substances, on organisms, biodiversity and ecosystems services. Nine model categories were inventoried: QSAR, dose-response, TKTD, SSD, food web, population, community, landscape and mixture models. They were developed for various species (terrestrial and aquatic vertebrates and invertebrates, aerial organisms, primary producers) and environmental compartments (soil, freshwater, seawater, air). These models are increasingly recognised for the regulatory risk assessment of pesticides but remain rarely used. The main limits of these models (for example, long-term effects are hardly considered) are discussed together with improvement avenues (multi-generational effects, multiple biotic and abiotic stressors...). This review also underlined a lack of models testing from field data and a lack of sensitivity and uncertainty analyses. Accurate modelling of the effects of pesticides and other stressors on living organisms, from their application in the field to their functional consequences on the ecosystems at different scales of time and space, would help going towards a more sustainable management of natural resources.

**5.03.V-02 Taking full advantage of modelling to better assess environmental risk due to xenobiotics**

***Sandrine Charles,*** *University Lyon, France*

In the European Union, more than 100,000 man-made chemical substances are awaiting an environmental risk assessment (ERA). Simultaneously, ERA of chemicals has now entered a new era. Indeed, recent recommendations from regulatory bodies underline a crucial need for the use of mechanistic effect models, allowing assessments that are not only ecologically relevant, but also more integrative, consistent and efficient. At the individual level, toxicokinetic-toxicodynamic (TKTD) models are particularly encouraged for the regulatory assessment of pesticide-related risks on aquatic organisms. In this paper, we first propose a brief review of classical dose-response models to put into light the on-line MOSAIC tool offering all necessary services in a turnkey web platform whatever the type of data to analyze. Then, we focus on the necessity to account for the time-dimension of the exposure by illustrating how MOSAIC can support a robust calculation of bioaccumulation factors. At last, we show how MOSAIC can be of valuable help to fully complete the EFSA workflow regarding the use of TKTD models, especially with GUTS models, providing a user-friendly interface for calibrating, validating and predicting survival over time under any time-variable exposure scenario of interest. Our conclusion proposes a few lines of thought for an even easier use of modelling in ERA.

**5.03.V-03 Improvements in toxicokinetic-toxicodynamic predictions of survival accounting for both time and concentration dependency - Towards innovative PBTKTD models**

***Sandrine Charles***[1]*, Jérémie Bruset*[1]*, Dominique Lamonica*[2]*, Ophelia Gestin*[3] *and Christelle Lopes*[1]*, (1) University Lyon, France, (2) Université Claude Bernard Lyon 1, France, (3) University of La Rochelle, France*

Regulatory authorities today recognize toxicokinetic and toxicodynamic (TKTD) models as a useful complement to current practice for risk assessment due time-dependent chemical exposure from the environment. Effect on survival can specifically be described by GUTS models (*General Unified Threshold model of Survival*) providing the survival probability versus both time and contaminant concentration. GUTS models depend on each species-substance combination, consisting of a TK part that relates exposure and internal concentrations within organisms; and a TD part that relates internal concentrations to the effects on survival. The TK part of GUTS models is today oversimplified with one compartment corresponding the whole organism, into which the contaminant enters and from which it leaves at a same rate simultaneously encompassing both accumulation and elimination. We will illustrate in this work how revisiting the TK part of GUTS models is likely to improve parameter estimates, especially in terms of reducing uncertainties. The original GUTS (model A) was compared to two alternatives: (model B) still with a single compartment but differentiating accumulation and elimination phases associated with two different rate constants; (model C) where an organism is subdivided into several compartments corresponding to target organs chosen on physiologically bases (PB) about the expected internal contamination pathway. This comparison was led on a common data set containing bioaccumulation and survival data from males of the sentinel species *Gammarus fossarum* exposed to several concentrations of Cadmium for 7 days. The bioaccumulated concentration in the whole body and in each organ, as well as the number of survivors were monitored at regular time points. Using also additional data sets, we finally demonstrate a real added value of using full PB-GUTS models, that become a little bit more complex (with a higher number of parameters to estimate) but reveal much more relevant for ERA (with better fits and clearly reduced uncertainties) as those today recommended by regulatory authorities.

## 5.04.P Bioaccumulation of Difficult to Test Substances - Advances in Methodologies and Risk Assessment Tools

### 5.04.P-Th090 Measurement of Octanol-Water and Butanol-Water Partitioning Coefficients for Relevant Per- and Poly-fluorinated Alkyl Substances.

***Henry Sluka**[1], Tyler Shonrock[2], Lawrence Burkhard[3], Ian Mundy[3], Will Backe[3] and David Mount[3], (1) Oak Ridge Institute for Science and Education (ORISE) participant at U.S. Environmental Protection Agency, (2) Oak Ridge Associated Universities, (3) U.S. Environmental Protection Agency*

Per-and-poly-fluorinated alkyl substances (PFASs) are a diverse class of synthetic compounds found ubiquitously in the environment and are shown to cause toxic effects in aquatic organisms. Solvent-water partitioning coefficients, such as octanol-water ($K_{ow}$), are fundamental properties that are widely used to predict or model environmental fate, bioaccumulation, and toxicity. However, agreement between solvent-water partitioning coefficients for PFASs derived from different computational methods is poor, owing to the challenges of modeling chemicals that are fluorinated and often ionized at ambient pH. EPA's CompTox Chemical Dashboard reports Log $K_{ow}$ values from various models for perfluorooctane sulfonamide (PFOSA), ranging from 5.02 (OPERA) to 7.64 (ACD/Labs) with similar or greater variability observed for other common PFASs. High-quality measured $K_{ow}$ values for PFASs are also rare, leaving a critical data gap that prevents evaluation of model performance. To address this data gap, we are experimentally measuring $K_{ow}$ values for a range of PFASs within the homologue series of carboxylic acids, sulfonic acids, sulfonamides, and fluorotelomer sulfonates. Measurements are made by OECD Slow Stir Method 123, with quantitation of both aqueous and organic phases by HPLC-MS and/or HPLC-MS/MS. In addition to directly measuring $K_{ow}$, we are also exploring the use of the butanol-water partition coefficient ($K_{bw}$) as a predictor of $K_{ow}$, a relationship that has proven robust for a number of other chemical classes but has not yet been unexplored for PFASs. Measurement of $K_{bw}$ as a predictor of $K_{ow}$ has some advantages, including faster equilibration kinetics, and easier quantification in the water phase due to butanol being less hydrophobic compared to octanol.

### 5.04.P-Th091 Mechanistic insights of cyclic volatile methyl siloxanes using physicochemical properties, bioavailability, metabolism and toxicity profiles

*Kathleen Plotzke[1], **Jaeshin Kim**[1], Kent Woodburn[1], Ellen M. Mihaich[2], Shihe Xu[1], Gary Kozerski[1] and Debra McNett[1], (1) The Dow Chemical Company, (2 )ER2*

Silicon chemistry is fundamentally different from carbon chemistry. Silicon is one period lower than carbon in the periodic table of the elements; therefore, silicon has a greater capacity than carbon to share electrons with oxygen. This difference is evidenced by the stronger bond (higher bond energies, higher bond angles, and shorter than expected bond lengths) associated with the silicon-oxygen bond as compared to the carbon-oxygen bond. The nature of the silicon-oxygen bond makes siloxane molecules, which results in weak interactions between other siloxane molecules. This is illustrated by the lower surface tension, viscosity and vapor pressure compared to hydrocarbons of similar molecular weight. Combined with their large size (10 atoms per Me2SiO unit) and only a moderate ability to accept hydrogen bonds, these fundamental characteristics of the cyclic volatile methyl siloxanes also lead to differences in the ability of these siloxanes to interact with each other and as solutes with environmental "solvents" or media such as water, organic carbon in soil/sediment, and lipids in biota, compared to traditional hydrophobic organic contaminants. These differences lead to a different combination of solubility, partitioning, bioaccumulation and reactivity properties that influence their distribution, fate and potential toxicity in the environment. These differences and this type of chemistry was not taken into account when the single media criteria were put into place to predict substances that could cause widespread contamination, would be difficult to reverse or remove from environmental media and could bioaccumulate. This presentation provides a review of the chemical structures, physicochemical properties, bioavailability, metabolism, toxicokinetic and toxicity profiles that suggest that the unique aspects of the chemistry must be taken into account when assessing properties such as persistence, bioaccumulation and environmental risk for this class of compounds.

**5.04.P-Th092 In situ gut bolus method development for dosing filter and non-filtering feeding fish**

***Gavin Saari**, Blake W. Sauey, Linnea M. Thomas and James J. Wamboldt, U.S. Geological Survey*

The evaluation of chemical bioaccumulation is essential to determine the risk of environmental pollutants but also during management chemical (i.e., pesticide) development. Due to the global transfer of aquatic biota outside their native geographical range resource managers utilize multiple control technologies (e.g., pesticides) for fisheries management and removal of nuisance fishes (e.g., invasive). Antimycin-A (ANT-A) is a historic ionizable management chemical, previously registered in the United States, that has been extensively studied and used for fish removals. Standard gavage methods for testing oral toxicity and bioavailability are not possible with filter feeding fishes such as Silver Carp and Bighead Carp (i.e., Bigheaded Carp) due to their physiology (e.g., pharyngeal teeth, lack of a true stomach, fragile GI tract). Due to ANT-A's non-selective toxicity, oral delivery systems that dose fish by exploiting filter feeding behavior (e.g., bigheaded carp) have been developed and demonstrated to enhance species selectivity of ANT-A. While lethality was observed in target species (i.e., Bigheaded Carp), oral delivery system optimization appeared necessary to improve dosing effectiveness and chemical bioavailability within the intestinal tract. Therefore, this presentation will discuss an in situ gut bolus method developed to examine oral delivery system effectiveness (e.g., toxicity, bioaccumulation) across the intestinal tract of filter feeding and non-filter feeding fish. Detectable chemical uptake across the gut was observed following chemical exposure. Gut bolus injections of liquid and encapsulated ANT-A were 100% lethal (4–30-h duration) with no mortality in control (blank) fish. The gut bolus approach aims to examine chemical uptake across the intestinal tract, characterize tissue biodistribution, and delivery system efficacy in fish. The developed method demonstrates an additional approach for fish bioaccumulation research with high log KOW compounds, such as ANT-A (log KOW = 5.1), to understand dietary based toxicokinetics for difficult substances, including management chemicals developed for fish removal.

## 5.06 Continuing Discussions on Incorporating Climate Change Model Predictions into Ecological Risk Assessments

**5.06.T-01 Concept for the 2022 SETAC Pellston Workshop on Integrating Climate Change Predictions into Ecological Risk Assessment**

*Ralph G. Stahl Jr.[1], Alistair Boxall[2], **Kevin Brix**[3], Wayne G. Landis[4], Jannicke Moe[5] and Jennifer Stauber[6], (1) DuPont (retired), (2) University of York, United Kingdom, (3) EcoTox LLC, (4) Western Washington University, (5) Norwegian Institute for Water Research (NIVA), Norway, (6) CSIRO, Australia*

A previous SETAC Pellston Workshop on Climate Change was held nearly 10 years ago, and resulted in seven publications that appeared in *Environmental Toxicology and Chemistry*. Since then additional reports by the Intergovernmental Panel on Climate Change (IPCC) have been published, and continue to express alarm about the lack of substantive actions by many nations to reduce their climate-forcing chemical emissions. In addition, the potential interaction of chemicals with climate change continues to be given little consideration, an oversight we noted 10 years ago and which stimulated our first workshop. The concept for conducting a second Pellston workshop on climate change and ecological risk assessment resulted from discussions among several of us about the need for revisiting the topic, and addressing some of the issues we did not address before. One issue we did not address included the ability to access climate models that could be scaled appropriately for an ecological risk assessment. Another was having members of the climate change modelling community interact with us directly, to provide the expertise in understanding and applying the models at scales useful for an ecological risk assessment. In this overview we will go into more details about the topics covered by the participants in the SETAC Pellston Workshop on Integrating Climate Change Model Predictions into Ecological Risk Assessments, held 20-24, June, 2022, at the Oscarsborg Fortress, outside Oslo, Norway. Additional presentations will be made by each of the three work groups developed for this workshop. At this time, at least four manuscripts from the workshop are expected to be completed in early 2023, and submitted to *Integrated Environmental Assessment and Management.*

**5.06.T-02 The Integration of Climate Change into the Problem Formulation Stage of Ecological Risk Assessments and Future Implications**

***Wayne G. Landis***[1], *Jannicke Moe*[2], *Rasmus Benestad*[3], *John F. Carriger*[4], *John Daniel Hader*[5], *Taro Kunimitsu*[6], *Rory Nathan*[7], *Rik Oldenkamp*[8] *and Andy Pitman*[9], *(1) Western Washington University, (2) Norwegian Institute for Water Research (NIVA), Norway, (3) Norwegian Meteorological Institute, Norway, (4) U.S. Environmental Protection Agency, (5) Stockholm University, Sweden, (6) Center for International Climate Research, Norway, (7) The University of Melbourne, Australia, (8) Radboud University Nijmegen, Netherlands, (9) University of New South Wales, Australia*

The problem formulation stage of an ecological risk assessment defines the scope of the analysis by delineating the ecosystems at risk, the characteristics of the stressors, the assessment and measurement endpoints, the exposure pathways between the stressor source and endpoints, and the risk hypotheses. It has become clear that the overarching effects due to global climate change can affect each of these components. Within the SETAC Pellston workshop on global climate change and ecological risk assessment, the integration of climate model projections into the prediction of near- and long-term risk was evaluated. A major impetus for this work is that climate change can affect ecological risks in multiple ways, including changing the use of chemical stressors such as pesticides, changing the use of a habitat by species of concern, and altering habitat quality. State-of-the-art global climate modelling and downscaling techniques may enable climate projections at scales appropriate for the study area. However, it is also important to realize the limitations of individual climate models and evaluate the uncertainties associated with these models in conjunction with the uncertainties of the traditional risk assessment pathways. Completed risk assessments that utilized Bayesian networks and represent advances in the science for their applications, were drawn upon for better predicting future risks to ecosystems. The tools and considerations developed from this working group may be helpful for elucidating the multiple future challenges to ecosystems and for better managing risks from a changing climate. The views expressed in this presentation are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

**5.06.T-03 Integrating Global Climate Change into Ecological Effects Assessment and Risk Characterization: Outcomes from a SETAC Pellston Workshop**

***Kevin Brix***[1], *Jennifer Stauber*[2], *Raoul-Marie Couture*[3], *Sophie Mentzel*[4], *Jason Rohr*[5], *Paul van den Brink*[6], *Julie Verheyen*[7] *and Jannicke Moe*[4], *(1) EcoTox LLC, (2) CSIRO, Australia, (3) Laval University, Canada, (4) Norwegian Institute for Water Research (NIVA), Norway, (5) University of Notre Dame, (6) Wageningen University & Research, Netherlands, (7) KU Leuven, Belgium*

From a global perspective, climate change (GCC) is currently the most important anthropogenically-driven environmental stressor. Chemical, physical, and biological stressors can interact with GCC at multiple temporal, spatial and biological scales of organization presenting a complex system in which to make projections of future ecological risk. Historically, climate change models operated at relatively large spatial and temporal scales but have increasingly become capable of making projections at scales that are more generally useful in regional and even local ecological risk assessments. Still, challenges remain in translating the outputs of climate change models into inputs for ecological risk assessment models. Here, we present some of the findings from a recent SETAC Pellston workshop. Specifically, we focus on potential approaches to integrating projected changes in climate-related stressors into an ecological risk assessment framework that considers direct interactions with ecological receptors, interactions with chemical, physical and biological stressors, as well as their interactions (toxicant-induced climate sensitivity and climate-induced toxicant sensitivity). We first provide some generalized concepts and approaches that will be applicable across a wide range of ecological risk assessment scenarios. Then, we provide a case study of the Great Barrier Reef, where we used a Bayesian network framework to assess the integrated risk from GCC along with physical, chemical, and biological stressors to this high value ecological system.

**5.06.T-04 Integration of Climate Model Projections and Pesticide Application Scenarios for Probabilistic Risk Assessment with a Bayesian Network Approach**

*Jannicke Moe[1], **Sophie Mentzel**[1], Rasmus Benestad[2], John F. Carriger[3], John Daniel Hader[4], Taro Kunimitsu[5], Rory Nathan[6], Rik Oldenkamp[7], Andy Pitman[8] and Wayne G. Landis[9], (1) Norwegian Institute for Water Research (NIVA), Norway, (2) Norwegian Meteorological Institute, Norway, (3) U.S. Environmental Protection Agency, (4) Stockholm University, Sweden, (5) Center for International Climate Research, Norway, (6) The University of Melbourne, Australia, (7) Radboud University Nijmegen, Netherlands, (8) University of New South Wales, Australia, (9) Western Washington University*

We present a Northern European case study from the SETAC Pellston workshop in June 2022 on integration of global climate change (GCC) modeling into ecological risk assessment. In Northern Europe, GCC is expected to result in increased temperatures and precipitation. The changes in weather patterns are expected to increase the occurrence of crop pests such as weeds, fungal disease, and insect pests. Increased pest pressures can in turn be expected to alter agricultural practices such as the frequency and combination of pesticide applications. Additionally, GCC may potentially have more direct effects on the environmental exposure of pesticides through changes in the transport, fate, and degradation of pesticides.

A Bayesian network (BN) has previously been developed as a meta-model for incorporating future climate projections and pesticide application scenarios with data on toxic effects to support environmental risk assessment for streams in agricultural areas in Northern Europe. This BN model was initially parameterized for a Norwegian case study with predicted environmental concentrations from a process-based pesticide exposure model and species sensitivity distributions derived from toxicity tests data.

Within the Pellston workshop, we aim to improve the existing BN model by incorporating more recent and realistic climate change scenarios, a larger number of climate models, and better methods for regional downscaling. The exposure prediction model WISPE has already been calibrated for the case study area with information local conditions and chemical properties of the pesticides used. This model can now be run using alternative climate model projections, as well as more realistic pesticide application scenarios. Our experiences from this case study will aid efforts to better account for uncertainty related to climate change in exposure modeling, effect assessment, and risk characterization. The graphical display of the BN model approach can also aid communication of risk under climate change scenarios to stakeholders such as policy makers and regulators.

The views expressed in this presentation are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

### 5.06.T-05 Managing the Environmental Impacts of Chemicals in a Changing Climate

***Ralph G. Stahl Jr.***[1]*, Alistair Boxall*[2]*, Mariana Cains*[3]*, Alizée O.S. Desrousseaux*[2]*, Sverker Molander*[4]*, Andrea Critto*[5]*, Eugenio Molina*[6]*, Julia Louise Sussams*[7]*, Hanna Andrea Rother*[8] *and Wayne G. Landis*[9]*, (1) DuPont (retired), (2) University of York, United Kingdom, (3) National Center for Atmospheric Research, (4) Chalmers University of Technology, Sweden, (5) University of Venice, Italy, (6) University of Alhambra, Spain, (7) Department for Environmental Food & Rural Affairs, United Kingdom, (8) University of Cape Town, South Africa, (9) Western Washington University*

We plan to present preliminary results of Work Group 3, one of the three work groups slated to participate in the SETAC Pellston Workshop on *Integrating Climate Change Predictions into Ecological Risk Assessment,* to be held 20-24 June 2022 at the Oscarsborg Fortress, near Oslo, Norway. We plan to focus on evaluating existing international and national chemical management frameworks (POPs, REACH, TSCA, among others), and whether they include climate change as part of the management process. Our evaluation will include other chemical management schemes, at smaller scales – for a regional or catchment level, cities or specific sites, and for single chemicals.  We will explore what tools are available to allow chemical management evaluations in the context of climate change at these various scales, identify data or methodological gaps that need to be addressed, and list recommendations suitable for guiding potential changes to national and international chemical management frameworks.  More details will be presented at the November 2022 SETAC North America meeting.

### 5.06.T-06 Incorporating Climate Changes Scenarios to Understand Future Water Stress and Modeling Its Impact on Consumer Product Chemical Exposure to the Environment

***Raghu Vamshi***[1]*, Brenna Kent*[1]*, Scott Dyer*[1] *and Andrea Carrao*[2]*, (1) Waterborne Environmental, Inc., (2) Kao USA*

Increased demand from a growing human population coupled with expansive evidence of climate change have intensified stresses on water availability and supply. The number of regions experiencing water stress is increasing, and municipalities are grappling with this stress by investing in water conservation, reuse, and recycling technologies. These methods will enable freshwater to be used in water stressed areas, however – they may require innovations in consumer products that are dependent on water for their function and disposal. Water stress in the U.S. was examined by evaluating datasets considering historic, current, and future water availability and use scenarios. Inclusion of anticipated climate change events required datasets that incorporated scenarios of estimated future population and per capita water use with high spatial resolution for the U.S. These datasets captured predicted temporal trends for the years 2020-2050 and were integrated with EPA's Clean Water Needs Survey data, which represents municipal wastewater treatment plant infrastructure across the U.S. The consolidated information was used to develop current and future water use scenarios. The influence of future water use scenarios on down-the-drain chemical exposures were predicted by evaluating four consumer product chemicals with various phys/chem properties. Results from the modeling provided a quantitative forecast of the potential impacts of water stress on down-the-drain chemical exposure and potential risk. Incorporating spatial and temporal variation in water stress and its impact to the risk of chemicals in the environment, through the lens of future scenarios, provides a new dimension in the development of consumer products. Incorporating these issues into product development, now, will ensure that both consumers and the environment will be appropriately stewarded, especially considering future environmental challenges.

### 5.06.T-07 Effects of the UV Filter BP3 in the Yellow clam Amarilladesma mactroides under a Predicted Global Warming Scenario

***Camila Martins****, Fernanda Lopes and Micheli Rosa de Castro, Federal University of Rio Grande, Brazil*

The benzophenone-3 (BP3) is one of the UV filters mostly detected in coastal waters and sediments and its is highly bioaccumulative with the potential to cause adverse effects on the biota living in contaminated regions. Considering that contamination by BP3 occurs in a climate change scenario, marked by global warming, this study aimed to evaluate the combined effects of exposure to BP3, at an environmentally relevant concentration (1 µg/L), with temperature increasing predicted by IPCC (24 °C) on the hemocytes and digestive gland of the yellow clam *Amarilladesma mactroides*. Results indicate that glutathione (GSH) is directly involved in BP3 detoxification by conjugating with xenobiotics or by neutralizing ROS generated under BP3 exposure. The BP3 (1 µg/L) at 20 oC increased ROS and antioxidants in the digestive glands of clams, without causing significant effect on lipid peroxidation (LPO). On the other hand, at high temperature (24 oC), BP3 inhibited antioxidant defenses leading to a rise in LPO in the digestive gland; however, this condition increased hemocyte viability. This augment in hemocyte viability may be related to the increase in the immune response sinc they play a fundamental role in the defense system of invertebrates. The Integrated Biological Responses (IBR) approach show that high temperature suppresses the ability of the digestive gland of *A. mactroides* to react to the presence of BP3, as the highest score was observed in animals exposed to 1 µg/L of BP3 at 20 °C (9.35), while the lowest was observed in BP3 group at 24 °C (1.02). The set of biomarkers responded to realistic exposure circumstances, evidencing the stress posed by the contaminant combined with the temperature increasing.

**5.06.T-08 Increased Ecological Risk Posed by Pesticides Discharged from WWTPs During Drought Conditions**

***Jacelyn Rice-Boayue**[1], Sara Kamanmalek[2] and Denise Adjidjonu[1], (1) University of North Carolina at Charlotte, (2) Smith College*

Wastewater treatment plants (WWTPs) have been identified as sources of pesticide pollution, especially in urban areas. The pathways by which pesticides enter municipal wastewater systems include residential and outdoor urban sources such as pet products, contaminated drinking water, disposals, and others. These sources are transported through the sewershed and are treated by WWTPs before being discharged into receiving streams. However, conventional treatment methods are generally ineffective at pesticide removal. Studies have shown that pesticides persist after undergoing primary, secondary, and tertiary treatment processes. These pesticides that remain in WWTP effluent pose a threat to aquatic organisms within receiving streams when concentrations exceed toxicity thresholds set by the U.S. Environmental Protection Agency (EPA). Furthermore, pesticide instream concentrations are heightened during drought streamflow conditions. Here in this study, we evaluate WWTP contributions to pesticide loading in the state of California for a range of temporal streamflow conditions. An ArcGIS-based spatial analysis is performed to estimate pesticide concentrations in WWTP effluent receiving streams and compared to respective EPA aquatic health benchmarks. The results of this study highlight the significant contributory role that WWTPs play in potential ecological threats posed by pesticides, and the influence of streamflow conditions on the estimated risks.

**5.07 Data and Methods to Support Cumulative Risk and Impact Assessments**

**5.07.T-01 A Vision for Research on Cumulative Impacts at the U.S. Environmental Protection Agency**

***H. Christopher Frey**, U.S. Environmental Protection Agency*

President Biden's Executive Order 14008 elevated environmental justice on the policy agendas for all federal agencies, including the United States Environmental Protection Agency (EPA). Racial and income-driven environmental health disparities are the result of decades of economic, regulatory, and land-use policy decisions that produced an inequitable distribution of pollution and environmental degradation. Further, pollution and environmental degradation are paired typically with economic and social distress, imposing additional burdens on communities of concern. The cumulative impacts of chemical and non-chemical stressors, including longstanding health disparities exacerbated by racial and social injustice, affect individual and community health and wellbeing. In support of EPA's goals of eliminating disparities in environmental and public health conditions, the EPA's Office of Research and Development's (ORD) world-class scientists aim to advance the science regarding assessment of such cumulative impacts. Our research vision is to: improve scientific understanding of the interactive effects of exposure to multiple chemical and non-chemical stressors; incorporate participatory research methods to empower communities; and translate research to inform decisions. Our vision is based on an interdisciplinary approach that includes the physical, chemical, biological, and social sciences. ORD's research on cumulative impacts will inform decision-making regarding interventions at the federal, state, and local levels and will be responsive to the needs and concerns of frontline and underserved communities.

**5.07.T-02 Using NHANES Data to Quantify the Magnitude of Allostatic Load in Relation to Existing Risk Assessment Uncertainty/Variability Factors**

***William D Klaren**, Liz Mittal and William Rish, ToxStrategies, Inc.*

Recent publications have demonstrated that vulnerable communities have higher basal levels of stress markers compared to the general population. Within those investigations, large epidemiology datasets, e.g. NHANES, were used to develop a composite metric, i.e. allostatic load, representing the cumulative burden of chronic stress and life events. This information has been used to find demographic populations with higher allostatic load. While crucially important, it is currently unclear how best to incorporate this type of information into risk assessments. An important question is the extent to which standard uncertainty/variability factors incorporated into dose-response factors in a risk assessment account for enhanced susceptibility due to exposure to non-chemical stressors.

To understand the magnitude of the susceptibility caused by elevated chronic allostatic load (non-chemical stress), NHANES questionnaire data was used to define a cohort with indicators consistent with those used by U.S. EPA's EJScreen tool for identifying communities susceptible to disproportionate health risks from environmental exposure.  Once defined, the distributions of the allostatic indicators (serum albumin, body mass index, serum C – reactive protein, serum creatinine, diastolic blood pressure, glycated hemoglobin, systolic blood pressure, total cholesterol, and serum triglycerides), individually and combined, were compared to that of the general population. This comparison facilitates an evaluation of the potential use of allostatic responses observed in the subset of NHANES data for a vulnerable/susceptible community as a relative indicator of non-chemical stress. For example, an allostatic load index based on these allostatic indicators relative to the general population could be incorporated into a cumulative risk index.

**5.07.T-03 How many chemicals typically drive the risk in real environmental mixtures in aquatic environments?**

***Ismael M Rodea-Palomares**, Zhenglei Gao, Markus Ebeling and Arnd Weyers, Bayer CropScience LLC*

With thousands of chemicals used in modern societies for a myriad of purposes, it is logical to think that many chemicals end up in the environment mixing up, and potentially contributing to combined mixture effects. In the other hand ever expansive analytical programs with ever-decreasing limits of quantification detect an ever-increasing number of anthropogenic substances in the environment, apparently fulfilling this prophecy. An important aspect of chemical risk is that the mere presence of a substance does not preclude an existing risk for human health or the environment. The classical quote of Paracelsus "the dose makes the poison" applies to unintentional environmental exposures to multiple chemicals, and only chemicals at sufficiently high concentrations may contribute to combine mixture effects. This principle has been extensively studied in human health and ecological research. There has been some limited field-based evidence demonstrating that in most environmental samples, when a chemical risk concern is found, it is driven by one or a very limited number of substances. Still, there is interest in understanding how broadly applicable these conclusions are at different geographic scales (e.g., at regional or continental). To better understand this question, we performed a screening level chemical mixtures risk assessment at the continental scale in the EU combining the largest publicly available chemical monitoring database in the EU: the Waterbase Waterquality ICM database, with state-of-the-art ecological effects metrics (SSDs) and chemical mixture assessment methods. The investigation included more than 330 chemicals (across pesticides, pharmaceuticals, metals, and industrial chemicals), 4000 waterbodies in 22 EU countries. We found that most monitoring samples did not pose a chemical risk concern from single substances or mixtures, with the top 3 chemical risk drivers per sample contributing on average a 90% of all the risk per sample. Further, the first risk driver alone contributed on average a 50% of all the risk. We found that a very limited set of specific chemicals (15 chemicals), contributed most of the risk in the sites found at risk across the entire database. Our findings provide critical evidence on the prevalence, characteristics, and drivers of the chemical mixtures risk found in unintentional mixtures currently in EU freshwaters.

**5.07.T-04 Exposure to the green environment is associated with improved biomarker-based indices of health and aging in adults**

***Andrey I Egorov***, *Shannon M. Griffin, Jennifer N. Styles, Jo Klein, Jason Kobylanski, Lindsay Wickersham, Edward Hudgens and Timothy J. Wade, U.S. Environmental Protection Agency*

Background. Inadequate exposure to health-promoting natural environments exacerbates adverse effects of chronic stress and contributes to reduced life expectancy in disadvantaged populations. Biomarker-based composite indices of allostatic load, systemic inflammation, and biological aging enable quantitative assessment of pre-clinical health effects of environmental factors; these indices are known predictors of morbidity and mortality.

Methods. This observational study in 335 adults in the Raleigh-Durham-Chapel Hill metropolitan area in North Carolina measured biomarkers of immune, neuroendocrine, and metabolic functions in blood samples, and DNA methylation status in leukocytes from 117 of these individuals. Allostatic load and inflammation indices were calculated by summing biomarker values dichotomized at health or distribution-based cutoffs. Epigenetic age was calculated from DNA methylation data using previously published definitions.

Results. An interquartile range increase in tree cover within 500 m of residence was associated with 13% (2%; 23%) and 16% (4%; 27%) lower allostatic load and inflammation indices, respectively, and 1.8 (0.8; 2.7) years lower mean difference between epigenetic and chronological ages in individuals who spent at least 30 min outdoors daily, adjusting for demographic and behavioral data, obesity, chronic infections, and two-dimensional spline of geographic coordinates. In individuals with residential tree cover above the median, spending at least 3 hours outdoors daily was associated with 68% (52%; 83%) reduction in systemic inflammation compared to controls spending less than 30 minutes outdoors. This was not observed for low tree cover settings.

Conclusion. Residential greenness is inversely associated with allostatic load and epigenetic aging. This abstract does not represent EPA’s policy.

**5.07.T-05 Research on Chemical and Non-Chemical Stressors to Further Understand How Cumulative Impact Assessments Can Improve Children's Health**

***Nicolle S. Tulve***[1]*, Kent Thomas*[1]*, Jonathan Essoka*[1]*, Intaek Hahn*[1]*, Kim Lichtveld*[2]*, Sandra Utile-Okechukwu*[3] *and Jacob Donovan*[1]*, (1) U.S. Environmental Protection Agency, (2) University of Findlay, Finland, (3) Oak Ridge Institute for Science and Education (ORISE) participant at U.S. Environmental Protection Agency*

Research on how the interrelationships between chemical and non-chemical stressors impact the health, well-being, and quality of life of vulnerable groups throughout their lifecourse is fundamental to cumulative impacts research. The Total Environment framework can be used to address cumulative impacts research for priority public health concerns where the total burden of chemical and non-chemical stressors and their interactions affect these outcomes. Children are exposed to both chemical and non-chemical stressors at different lifestages throughout their lifecourse. Exposure to some chemical stressors (e.g., pesticides, metals, perfluorinated compounds) is routinely measured and assessed. Research on non-chemical stressors (e.g., poverty, climate change, extreme weather events, violence, food insecurity, lack of access to greenways and recreational facilities, inadequate health care) is less routine in the context for potential combined effects with chemical stressors. To advance our understanding of the relationships among chemical and non-chemical stressors in

support of cumulative impacts research, we need to understand how non-chemical stressors influence the biological response to a chemical exposure, ultimately affecting health and well-being. To do this, we have employed a multi-pronged approach including systematic literature reviews, meta-analyses, and analysis of secondary data. Our efforts are focused on two childhood public health challenges – obesity and neurocognitive health – with assessments showing that both are influenced by a myriad of stressors. Our presentation will expand on our methodological approach, results to date, and how a cumulative impacts research approach can be used to improve these outcomes. By understanding how health, well-being, and quality of life are influenced by our everyday environments, we can begin to understand what we can do to improve conditions for individuals living in overburdened communities.

**5.07.T-06 Visualization and Analysis of Cumulative Risks Related to Resilience from Climate Change-induced Flooding in the Great Lakes Watershed**

***Jeffrey Ashby*[1] *and Diane Henshel*[2]*, (1) Indiana University, Bloomington, (2) Indiana University***

Flooding events are becoming more frequent and intense due to climate change. Flood events can be seen as another exposure when looking at the cumulative risk to which people are exposed, especially those in which marginalized communities. Quantifying flood-associated risks in a cumulative risk assessment would better inform communities and governments in ways to increase their resilience to these climate-driven stressors. In this project, we are taking a spatial approach to cumulative risk assessment (CRA) to evaluate resilience in the face of flooding in the Great Lakes watershed. Our CRA takes many resiliency factors into account, including; 100-year, 500-year, and 1000-year floodplains, access to services such as fire stations, hospitals, emergency shelters, and emergency operations centers, the CDC Social Vulnerability indices, Environmental factors like excessive temperature, food insecurity, private wells, U.S. Census-derived socioeconomic indices, traditional infrastructure which include power stations, cell phone towers, as well as transportation networks, underground storage tanks, and vulnerable populations including tribal lands. All of these factors are analyzed in a geographic information system that uses 5km hexagons to standardize the discrepant geospatial elements. These hexagons allow us to categorize and combine the varied stressors into metrics that can then be mapped back to create an overall ecological risk map. The results of this CRA is an easily viewable map, and corresponding spreadsheet, which we will make accessible on an Internet portal. This portal will be a place where individuals and communities can get a better idea of their CRA and what stressors are contributing most to their vulnerability. This study will be a basis for future prediction of the impact of climate change-induced flooding in the Great Lakes watershed and will continue to be built upon by adding additional stressors.

**5.07.T-07 Improving Cumulative Risk Assessment in Fenceline Communities: A Case Study in Southeast Pennsylvania**

***Andrea Chiger****, Carolyn Gigot, Mary Fox, Ellis Robinson, Mina Tehrani, Kirsten Koehler, Ana Rule, Peter DeCarlo, Thomas Burke and Keeve Nachman, Johns Hopkins University*

Cumulative risk assessment (CRA) is key to accurately characterizing health risks in fenceline communities, which bear the brunt of environmental pollution and often face challenging socioeconomic conditions. Current methods for EPA's approach to CRA move beyond the traditional single-chemical approach to risk assessment by considering mixtures but typically only assess the most sensitive health endpoint for each chemical in the mixture. We developed an approach to characterize risk more holistically by drawing upon existing hazard information to evaluate multiple health effects for each chemical (i.e., considering not only the most sensitive endpoint, but also those occurring at higher doses). Our Hazardous Air Pollutant Monitoring and Assessment Project (HAP-MAP) served as a case study to demonstrate these approaches. In HAP-MAP, our transdisciplinary team used state-of-the-art measurement techniques at a fixed site and with a mobile laboratory to estimate fenceline community exposures to over 70 volatile organic compounds and metals, providing our exposure assessment with high spatial resolution and chemical complexity that would not be attainable using regulatory monitoring data such as EPA's Air Toxics Screening Assessment (AirToxScreen). To characterize combined risks of these exposures, we compiled hazard information on all available non-cancer health effect categories (e.g., cardiovascular, respiratory) using sources such as EPA's CompTox and Hazard Comparison Dashboard. Next, we employed a margin of exposure methodology to evaluate our exposure estimates from HAP-MAP based on identified hazard information. For each non-cancer health effect category, we calculated the inverse sum of the reciprocal margins of exposure across all chemicals to characterize risk. Comparing our approach using multiple health effects to an assessment focused on sensitive endpoints only, our results show that current methods likely underestimate health risks in fenceline communities. Similarly, risk estimates were far lower when based on estimated air concentrations from EPA's AirToxScreen instead of localized measurements from HAP-MAP, indicating the need for improved spatial and temporal monitoring in fenceline communities. Our work demonstrates the need for transdisciplinary collaborations and innovative approaches to improve the accuracy of risk assessment in fenceline communities.

**5.07.T-08 Discussion - Data and Methods to Support Cumulative Risk and Impact Assessments**
***William Rish**[1] and H. Christopher Frey[2], (1) ToxStrategies, Inc., (2) U.S. Environmental Protection Agency*

**5.08 Developing Endangered Species Assessments for Pesticides in the United States: Progress to Date and Next Steps**

**5.08.T-01 The United States Department of Agriculture's Role in the Endangered Species Act Consultation Process for Pesticides**
***Elyssa Arnold**, Cameron Douglass, Clayton Myers and Kimberly Nesci, U.S. Department of Agriculture*
The United States Department of Agriculture (USDA) is an important part of the federal Interagency Working Group (IWG) striving to improve the Endangered Species Act (ESA) section 7 consultation process for pesticide registration and registration review. USDA works closely with the Environmental Protection Agency (EPA) and the Fish and Wildlife and National Marine Fisheries Services to represent the perspectives of agricultural stakeholders during the development of EPA's Biological Evaluations (BE) and the Services' Biological Opinions (BO). USDA draws on established relationships with grower groups, proprietary usage data, and other tailored outreach efforts to pest management experts to gather critical data to inform the BE and BO development. Key information on (1) typical use practices, (2) specialized uses (e.g., for quarantine programs), and (3) the practical implications of proposed mitigations can ensure that mitigations are effective in protecting federally listed species and critical habitats, as well as implementable for agricultural pesticide users. The recent consultation for malathion with the Fish and Wildlife Service demonstrated the value of this approach. USDA's ongoing participation in the mitigation discussions contributed to a workable and effective set of Reasonable and Prudent Alternatives and Reasonable and Prudent Measures that also led to a No Jeopardy final opinion. By including the grower and other user perspectives early in the consultation process, USDA can help support an efficient process that results in reasonable, meaningful, and practical mitigation.

**5.08.T-02 Deriving Aquatic Exposure Distributions Using a Probabilistic Pesticide Usage Approach**
***Hendrik Rathjens**[1], Michael F Winchell[1] and Sean McGee[2], (1) Stone Environmental, Inc., (2) Bayer CropScience LLC*
Current methods used by the US EPA for evaluating the potential effects of pesticides on aquatic species protected by the Endangered Species Act include a probabilistic assessment of exposure for each species. The approaches applied by EPA in recent assessments account for variability in pesticide application timing, surface runoff potential, and usage data to make estimates on the magnitude and likelihood of exposure for each species. However, the assumptions used to derive likelihood and magnitude of exposure are not consistent (e.g., different application rates are assumed) and the resulting probabilistic exposure distribution does not account for the extent or likelihood of pesticide usage within an aquatic species range or critical habitat. This presentation will discuss a methodology to account explicitly for the impact of pesticide usage on exposure magnitude and likelihood when deriving a species-specific probability distribution of aquatic exposure. The methodology accounts for uncertainty in annual pesticide usage at the Crop Reporting District (CRD) level by

sampling from five different usage years, each receiving equal probability. The uncertainty in pesticide application rates and area treated is accounted for by assuming both maximum and minimum recommended annual application rates according to the pesticide label. Because field-specific pesticide application location information is unavailable, we applied a probabilistic spatial sampling approach to derive multiple random realizations of the locations of pesticide usage within a CRD at a one square mile resolution. In this assessment, 25 use footprints were developed for each usage year and rate scenario, resulting in a total of 250 use footprints realizations, providing a robust set of spatial pesticide use scenarios. The use footprints were intersected with watersheds representing static and flowing water body habitats within each species range or critical habitat. The intersecting watersheds were then simulated using EPA's regulatory modeling tools to derive comprehensive exposure distributions reflecting the variability and spatial uncertainty of pesticide usage locations across a broad spectrum of habitat locations. These exposure distributions could be used within EPA's current probabilistic assessment framework, providing a critically needed link between pesticide usage and the magnitude and likelihood of exposure for a species.

**5.08.T-03 Application of Landscape Scale Lines of Evidence in Pesticide Endangered Species Assessments to Promote Focused Avoidance and Minimization Measures**

***Matthew E. Kern**[1], Steve Kay[2], Christopher M. Holmes[3], James Cowles[4] and Kevin Henry[4], (1) Balance EcoSolutions LLC, (2) Pyxis Regulatory Consulting, Inc. / Generic Endangered Species Task Force (GESTF), (3) Applied Analysis Solutions LLC, (4) Tessenderlo Kerley, Inc.*

Protection of federally-listed threatened and endangered species requires pesticide risk assessments that are comprehensive enough to determine if jeopardy to a species or adverse modification to designated critical habitat is reasonably expected to occur. These assessments must also be refined to the extent that it is possible to identify if, and where, avoidance or minimization measures may be useful to address potential Jeopardy and minimize take to individuals. Growers and product users need solutions that allow them to protect species without restricting pesticide products based on incomplete risk assessment. Highly generalized assessments that do not account for species-specific landscape scale information do not provide adequate detail to accomplish this goal. Further, considering single lines of evidence within the assessment without considering the collective value of all lines of evidence can significantly limit the relevance and reliability of an assessment. Uncertainties about certain data, methods and analyses can be addressed by maintaining a holistic view of the entire analysis. Here we present examples of landscape level effects assessments and proposed mitigation measures that support a pragmatic and protective approach to the conservation of federally listed species. Species- and product-specific information are used to evaluate the likelihood of exposure, jeopardy and potential take such that avoidance and minimization measures can be highly targeted to where they may be needed.

**5.08.T-04 Species Extent in Regulatory Decision Making: Tracking Changes and Refinements to the Species Ranges**

***David Campana**[1], Ashlea R. Frank[1], Tilghman Hall[2], Lula Ghebremichael[3], Chris Hassinger[4], John Marton[5] and Paul Whatling[6], (1) Compliance Services International (CSI), (2) Bayer CropScience LLC, (3) Syngenta Crop Protection, (4) BASF Corporation, (5) Corteva Agriscience, (6) FMC Corporation*

Under current policy of the US Environmental Protection Agency (EPA), pesticides submitted for registration and registration review are assessed for their potential risk to endangered species, per guidelines set forth in the Endangered Species Act (ESA). EPA, as well as the US Fish and Wildlife Service and NOAA Fisheries (the Services), initially assess potential risk of exposure of a species to a pesticide by determining the extent of overlap between species range and potentially treated use sites. This approach can overestimate risk because some species ranges are not refined to include only those habitats suitable for their survival and/or areas where the species is likely to occur. In some cases, the extent of species range is defined by administrative boundaries, such as county and or state borders, representing potential gross overestimation of potential exposure. Using unrefined species range information to define areas where pesticide mitigation measures may be needed, or pesticide use limitation areas (PULAs), creates unnecessary restrictions in areas that are unlikely habitat for the species. This presentation discusses data that could be used to refine species range, such as documented habitat information and habitat locations, and provides examples of how refined species extents could feed into the current overlap and mitigation area analytical process. These proposed refinements of species range are not without precedent; we will introduce the FIFRA Endangered Species Task Force (FESTF) Gopher information management system as a tool to mine data and catalog refined species data that can be used as substitutes for range in overlaps and PULAs. Regulatory challenges, including tracking changes in data/versions over time, that can be minimized by Gopher will also be discussed.

**5.08.T-05 Evaluating Effects of Pesticide Use to Federally Listed Species and its Practical Application in Recent Pesticide Registration Actions**

***Austin Wray*** *and Joshua Antoline, U.S. Environmental Protection Agency*

In January, the Environmental Protection Agency (EPA) released a workplan that described the Office of Pesticide Programs' plans to address its Endangered Species Act (ESA) obligations during pesticide registration. As part of this commitment, the Environmental and Fate Effect Division (EFED) is developing a new approach to evaluate the potential effects of registered or proposed pesticide uses on federally listed, endangered and threatened ("listed") species and their designated critical habitats. The new approach adopts methods employed by the Fish and Wildlife Service and National Marine Fisheries Service (hereafter referred to as the Services) in their Biological Opinions. EPA's effects determinations continue to predict whether a pesticide has no effect or may affect an individual of a listed species or critical habitat. Where a pesticide may affect an individual, EPA assesses if the pesticide is likely or not likely to adversely affect an individual or the critical habitat. When a likely to adversely affect determination is made, EPA predicts whether it is likely or not that a species or critical habitat will be jeopardized or adversely modified (J/AM). When changes to the action are needed to avoid J/AM or minimize take, EPA evaluates the effectiveness of those changes on reducing exposure. This presentation will provide an overview of EPA's evolving effects determination approach using an example of a recent effects determination.

**5.08.T-06 Challenges and opportunities for using population modeling to assess risks of pesticides to threatened and endangered species.**

***Valery Forbes***[1] *and Sandy Raimondo*[2]*, (1) University of Minnesota, (2) U.S. Environmental Protection Agency*

Although population modeling has a long history as a decision support tool in conservation and fisheries biology, its use to inform ecological risk assessments (ERA) has been limited. Advances in methodology, publication of numerous case studies, and improved guidance in model development, documentation, and evaluation demonstrate the value of population modeling over traditional effects assessments that are largely based on individual-level, toxicological responses of standard test species. Whereas the European Union has been slowly moving forward to incorporate population models and other mechanistic effect models in pesticide risk assessments, US government agencies have not. This presentation will discuss recent advances in Population modeling Guidance, Use, Interpretation, and Development for ERA (Pop-GUIDE), particularly in the context of assessing risks of pesticides to threatened and endangered species in a scientifically defensible manner. We will highlight recently published models for threatened and endangered species that have followed Pop-GUIDE, discuss challenges in expanding the use of population models for listed species decision support, and recommend steps to move their application forward.

**5.08.T-07 Why, When, and How to Incorporate Usage Data in ESA Pesticide Risk Assessments**

***Michael F. Winchell***[1]*, Scott Teed*[2] *and Hendrik Rathjens*[1]*, (1) Stone Environmental, Inc., (2) Intrinsik Corp., Canada*

Pesticide usage data and other lines of evidence can be used to reasonably estimate the likelihood of and magnitude of exposure for listed species evaluated in pesticide biological evaluations and biological opinions. Recent biological evaluations produced by the EPA for the carbamates, herbicides, and neonicotinoids applied usage data in quantifying the likelihood of exposure based on an overlap analysis approach. In situations where uncertainty is high (e.g., indistinct spatial data for specialty vegetables etc) conservative assumptions are made to account for this uncertainty. Usage data, when available and sufficiently robust, must be used in a listed species risk assessment for pesticides in keeping with the ESA 'best available data' standard and the requirement to make reasonable and realistic exposure assumptions. In some cases, pesticides may have insufficient usage data available, with some new active ingredients potentially having none. A decision tree may assist in determining when, how, and why usage data should or should not be applied. This presentation will provide an overview of the usage data sources that should be considered, including uncertainties in those data sources. Brief case studies will be provided as evidence that the interpretation of overlap and likelihood of exposure is in fact highly influenced by usage information through a series of quantitative examples. Additional analysis will be presented regarding the importance of considering usage data when estimating the *magnitude* of exposure as opposed to only considering exposure magnitudes derived from modeled edge-of-field receiving waters assuming usage data.

**5.08.T-08 Let's Keep It Local: Refining Aquatic Exposure Estimates Using Species-Specific Landscape Information**

***Christopher M. Holmes**[1], Steve Kay[2], Matthew E. Kern[3], Logan Insinga[1], Dana Christian[1], Jim Cowles[4] and Kevin Henry[4], (1) Applied Analysis Solutions LLC, (2) Pyxis Regulatory Consulting, Inc. / Generic Endangered Species Task Force (GESTF), (3) Balance EcoSolutions LLC, (4) Tessenderlo Kerley, Inc.*

Prospective aquatic exposure modeling is one of several tools used to assess the potential for impacts in Biological Opinions (BO) for pesticides. The Biological Evaluation (BE) prepared by the EPA for the insecticide carbaryl provides aquatic exposure estimates by using well-established pesticide fate and transport models (e.g., PRZM, VVWM, AgDRIFT) applied to a standardized set of crop/soil/weather scenarios with reported aquatic concentrations generally limited to 30 annual maxima values. Variability in aspects such as spatial proximity, the incorporation of Percent Crop Area, distribution of pesticide treated acres, and temporal aspects of exposure have been identified as areas for possible refinement in recent BEs and BOs and are employed to better inform the risk evaluation. This paper describes a highly efficient, structured approach that builds on the EPA's aquatic modeling to increase the spatial/temporal context and resolution of exposure estimates to listed species and produces well-defined and reproducible species-specific estimated aquatic concentrations. Utilizing data for each species, the set of NHD+ catchments, landscape-based crop proximity and density, pesticide usage (e.g., Percent Crop Treated), and additional user-selected options (e.g., PCT multiplier, PCA multiplier, alternate application rates), inputs were processed to produce refined exposure concentrations suitable for aggregation at multiple spatial scales appropriate for the species being examined (e.g., range, critical habitat, spawning areas, recovery areas, existing conservation programs). Temporal aspects were examined using multiple endpoints of the 30-year dataset (e.g., 1-in-15 year, median of annual maxima, $95^{th}$ percentile of daily) as well as daily distributions. Individual PWC output files (labeled use/HUC/aquatic bin/proximity/application method) were scanned using Python scripts to develop summary concentration profiles for differing time periods and distribution points to provide inputs for the refined modeling. The landscape-scale aquatic modeling results provide valuable information to help inform localized mitigations for individual species and across specific use patterns.

**5.08.P Developing Endangered Species Assessments for Pesticides in the United States: Progress to Date and Next Steps**

**5.08.P-Th093 Estimating Risk for Butterflies Exposed to Permethrin Applied by Ultra-low Volume Sprays**

***Tim Bargar**, U.S. Geological Survey*

The U.S. Fish and Wildlife Service has designated 31 butterfly species as either threatened or endangered. Several of these listed species reside in southern Florida (Florida leafwing [*Anaea troglodyta floridalis*], Bartram's scrub hairstreak [*Strymon acis bartrami*], Schaus swallowtail [*Heraclides aristodemus ponceanus*], and Miami blue butterflies [*Cyclargus thomasi bethunebakeri*]) and are potentially exposed to mosquito control insecticides due to routine ultra-low volume (ULV) sprays in residential areas. We have been investigating various factors affecting permethrin risk for butterflies to understand risk of the ULV sprays to the listed species. First is the possible role of butterfly movement on exposure to pesticides applied by ULV sprays because movement (flying) enhances mosquito exposure during ULV sprays. Live (moving) and dead (not moving or roosting) butterflies were exposed to permethrin during five ULV sprays and then analyzed for permethrin. Permethrin concentrations were higher on live butterflies (81 – 1,288 ng/g ww) than on dead butterflies (36 – 758 ng/g ww) in 4 of the 5 sprays conducted so far indicating movement during ULV sprays may also enhance butterfly exposure. Second, exposure of adult butterflies and live mosquitoes to permethrin after 3 ULV sprays was compared to understand relative exposure. Caged, co-located butterflies and mosquitoes were deployed at 3 distances (50', 150', and 250') from the spray truck during each of 3 sprays. Concentrations on mosquitoes (BD – 18,124 ng/g ww) were typically higher than on butterflies (BD – 4,247 ng/g ww) indicating mosquitoes would experience greater exposure during ULV sprays. Last, triplicate dose response studies for each of 2 mosquito species (*Culex quinquefasciatus* and *Aedes aegypti*) were conducted to determine LD50s for comparison with published LD50s for adult butterflies. The LD50s for the mosquitoes (81 and 166 ng/g ww) were lower than published LD50s for adult butterflies (180 – 1,100 ng/g ww) indicating mosquitoes may be more sensitive to permethrin than adult butterflies. While data from the above studies indicate mosquitoes may experience greater risk during ULV sprays (enhanced exposure and more sensitive), adult butterfly exposure (moving and non-moving) was sufficient to present a significant risk. Additional studies are underway to evaluate the utility of pesticide residues on leaves for estimation of butterfly exposure to the pesticide.

## 5.08.V Developing Endangered Species Assessments for Pesticides in the United States: Progress to Date and Next Steps

### 5.08.V-01 US EPA's Approaches to Determining Effects of Pesticides on Endangered and Threatened Species

***Kristina V. Garber****, U.S. Environmental Protection Agency*

The Endangered Species Act directs federal agencies to ensure that their actions are "not likely to jeopardize the continued existence of any endangered species or threatened species or result in the destruction or adverse modification of habitat of such species." In regard to pesticide products, the US Environmental Protection Agency's actions involve the registration of new active ingredients or uses or the re-evaluation of existing pesticides. EPA's process for assessing potential effects of pesticide related actions on listed species has changed over the years. This presentation will provide a brief overview of EPA's evolving method, which has largely focused on assessing effects to individuals of listed species. Based on approaches used by the US Fish and Wildlife Service and National Marine Fisheries Service, EPA is currently developing a method to predict which species and critical habitats may be jeopardized or adversely modified (J/AM) by pesticide actions. This can allow for identification and evaluation of changes to the action to avoid potential J/AM. This presentation will discuss EPA's current approaches to predicting potential individual-level and species-level effects of pesticide actions.

### 5.08.V-02 Incorporation of Conservation Measures into Pesticide Consultations under the Endangered Species Act

***Rosemary Burk*** *and Nancy Golden, U.S. Fish and Wildlife Service*

The U.S. Fish and Wildlife Service has been working with federal agency partners, pesticide registrants, and stakeholder groups as we engage in Endangered Species Act section 7 consultations for pesticide registrations under FIFRA. Due to the nature and often national scale of these registrations, there is potential for numerous threatened and endangered species and their critical habitats to be exposed to pesticides as labeled and used. Identifying and mitigating for these effects early in the consultation process can result not only in a more efficient consultation process, but greater protection for listed species and their critical habitats through timely implementation of conservation measures. To facilitate the incorporation of protective measures into the consultation process, we are exploring ways to use available science to consistently describe and analyze the effectiveness of various measures in reducing exposure and effects of these chemicals to listed species and critical habitats. The value of any particular conservation measure may vary depending on characteristics of the listed species requiring consideration such as where the species resides, what habitat(s) it can be found in, and its route of exposure to the pesticide. In addition, the feasibility of implementing a particular conservation measure may be influenced by the crop or application site of concern and the geographic area of application. We will discuss progress, challenges, and areas of potential collaboration for developing methodologies or filling important data gaps where science is currently lacking to measure the effectiveness of conservation measures in reducing exposure and effects to nontarget species.

**5.08.V-03 A Probabilistic Approach for Chronic Effects Assessments using Acute to Chronic Ratios Distributions in a Vernal Pool Case Study**

***Leah Oliver****, Sumathy Sinnathamby, Tom T. Purucker and Sandy Raimondo, U.S. Environmental Protection Agency*

Some pesticides are designed to cause lethality to pest species via physiological pathways that are also shared by nontarget species for which toxicity data is usually available only for a limited number of species, creating a need for robust methods to estimate acute and chronic toxicity with minimal data. We used a unique probabilistic approach to estimate risk of chronic effects of 2 organophosphate (OP) pesticides on the vernal pool fairy shrimp *Branchinecta lynchi*. Acute toxicity estimates were derived from Monte Carlo (MC) sampling of acute toxicity distributions developed from interspecies relationships using surrogate species. Within each MC draw, acute values were divided by an acute to chronic ratio (ACR) sampled from a distribution of ACRs for organophosphate (OP) compounds and invertebrates, producing a distribution of chronic effects concentrations. Probability of risk was characterized using MC simulations of exposure concentration distributions representing four seasonal scenarios. A deterministic chronic effects threshold was compared with the probabilistic method, demonstrating the deterministic estimate of chronic toxicity under-estimated chronic risk for two OPs, chlorpyrifos and diazinon. Demonstration of this approach for a focal species, the fairy shrimp *B. lynchi*, expands potential interpretation of risk to the vernal pool community level. By coupling a novel probabilistic approach for estimating chronic effects using ACRs with probabilistic exposure distributions, risk characterization that harnesses uncertainty in both exposure and effects is possible and applicable to higher-tiered ecological assessments.

**5.08.V-04 Sturgeons are biodiversity priorities needing special protection from chemicals and waste**

***Zihan Xu***[1]*, Fengchang Wu*[2]*, Ying Wang*[3]*, Kenneth M.Y. Leung*[4]*, Karen Kidd*[5] *and Bryan W. Brooks*[6]*, (1) Beijing Normal University, China, (2)Chinese Research Academy of Environmental Sciences, China, (3) Beihang University, China, (4) City University of Hong Kong, China, (5) McMaster University, Canada, (6) Baylor University*

Acipenseriformes (sturgeon and paddlefish) belong to a group of ancient fish that are broadly distributed throughout the Northern Hemisphere and often described as "living fossils" as they have precious biological germplasms and hereditary resources. Twenty-three of these unique organisms are threatened with extinction risk; among them, 17 species are specifically facing an extremely high risk of extinction. The worldwide sturgeons are declining mostly due to overfishing, habitat degradation, river regime shifts and migration interruption; however, as highlighted by the recent Global Chemicals Outlook II assessment from the United Nations Environment Program, extensive input of chemicals and waste, including endocrine disrupting chemicals (EDCs), into the aquatic environment pose underappreciated risks to aquatic organisms and ecosystems, and threaten the progress for achieving many of the Sustainable Development Goals. Building on previous efforts documenting the toxicity profiles of chemical contaminants on aquatic organisms, we examined toxicology information for sturgeons because even at very low concentrations, biologically active chemicals such as EDCs may adversely affect sturgeons and contribute to their worldwide decline. This analysis developed species sensitivity distributions (SSDs) and found that sturgeons are among the most sensitive aquatic species to five select representative EDCs with an overall proportion below 20% on the SSD curves. In particular, the Acipenser ruthenus was the most sensitive species to bisphenol A and tetrabrombisphenol A with the species proportion lower than 2% and 3% respectively, on the respective SSDs. We also performed a statistical analysis of the distribution of Acipenseriformes based on Fishbase, which is consistent with key priority habitats of endangered sturgeons identified in the World Wildlife Fund (WWF) Network Sturgeon Strategy 2017. It shows that EDCs and other contaminants have been detected in key sturgeon habitats, and exposure to a number of chemicals in some of these river basins are relatively high. Further, exposure distributions of the selected chemicals overlap with the chronic toxicity endpoint of the Acipenseriformes, which indicate apparent risks exist for this vulnerable group of species. Herein we recommend that effective management of chemicals and waste, including EDCs, is needed to reduce further impacts on these very old species.

## 5.09 Environmental Impact of Currently Marketed Sunscreens and Potential Human Impacts of Changes in Sunscreen Usage

### 5.09.T-01 Introduction to the Report by the National Academies Committee on Environmental Impact of Currently Marketed Sunscreens and Potential Human Impacts of Changes in Sunscreen Usage

***Emily Twigg**, National Academies of Sciences, Engineering, and Medicine*

Concerns have been raised about the potential toxicity of sunscreens to a variety of marine and freshwater aquatic organisms, particularly corals. At the same time, there are concerns that people will use less sunscreen as a result of environmental concerns. In 2021, an ad hoc committee of the National Academies of Sciences, Engineering, and Medicine was established to review the state of the science on the use of UV filters currently marketed in sunscreens, including their fate and effects in aquatic environments and the potential public health implications associated with reduced use. An ad hoc committee composed of members representing experience from numerous sectors—academia, non-profit, industry, and government—completed its report in 2022. This presentation provides an overview of the National Academies process, describes the context and specific statement of task for this report, and outlines the committees approach to this task. A summary of the main overarching findings and recommendations from the report will also be provided.

### 5.09.T-02 Physical-Chemical Properties, Sources, and Fate of UV filters in Aquatic Environments

***Christopher Higgins**, Colorado School of Mines*

An ad hoc committee of the National Academies of Sciences, Engineering, and Medicine reviewed the state of the science on the use of the active ingredients in sunscreens (UV filters) currently marketed in the United States. This review was conducted to provide information useful for future application in ecological risk assessments, by reviewing information on UV filter fates, exposure, and effects. This presentation provides an overview of the problem formulation step of an ecological risk assessment, where the physical-chemical profiles of the UV filters are reviewed, in addition to information relating to the identification of sources and their fate in aquatic environments. A summary of the committee's findings, conclusions, and knowledge gaps related to sources and fate characteristics is included in the presentation.

### 5.09.T-03 Exposure Analysis of UV filters in the Aquatic Environment and in Biota

***Charlie Menzie**[1] and Scott Belanger[2], (1) Exponent, (2) Procter & Gamble Company (retired)*

An ad hoc committee of the National Academies of Sciences, Engineering, and Medicine reviewed the state of the science on the use of the active ingredients in sunscreens (UV filters) currently marketed in the United States. This review was conducted to provide information useful for future application in ecological risk assessments, by reviewing information on UV filter fates, exposure, and effects. This presentation describes the committee's analysis of the available exposure and bioaccumulation data. This analysis summarized the state of the science on what is known about the relative quantities of UV filters entering the variety of aquatic environments (e.g., estuary, lake, coral reef) and well as measured concentrations in these environments and the biota. This also identified potential routes of exposure to UV filters and the potential for bioaccumulation in aquatic organisms. A summary of the committee's findings, conclusions, and knowledge gaps related to exposure is included in the presentation.

**5.09.T-04 Toxicity of Organic UV Filters in Aquatic Ecosystems**
***Carys Louise Mitchelmore****, University of Maryland Center for Environmental Science*
An ad hoc committee of the National Academies of Sciences, Engineering, and Medicine reviewed the state of the science on the use of the active ingredients in sunscreens (UV filters) currently marketed in the United States. This review was conducted to provide information useful for future application in ecological risk assessments, by reviewing information on UV filter fates, exposure, and effects. This presentation will summarize the state of the science on what is known about the potential effects of organic UV filters on aquatic organisms, including the potential for endocrine disruption, photo-activation, and other reported effects on molecular, cellular, organismal, population, community-level, and ecosystem-level endpoints. The committee's report also focused on identifying any potential Endangered Species Act listed organisms or those that are the subject of targeted management plans that have a likelihood of exposure. A summary of the committee's findings, conclusions, and knowledge gaps related to effects from organic UV filters is included in this presentation.

**5.09.T-05 Toxicity of the Inorganic UV Filters Zinc Oxide and Titanium Dioxide in Aquatic Ecosystems**
***Rebecca Klaper****, University of Wisconsin, Milwaukee*
An ad hoc committee of the National Academies of Sciences, Engineering, and Medicine reviewed the state of the science on the use of the active ingredients in sunscreens (UV filters) currently marketed in the United States. This review was conducted to provide information useful for future application in ecological risk assessments, by reviewing information on UV filter fates, exposure, and effects. This presentation summarizes what is known about the potential effects of the inorganic UV filters, zinc oxide and titanium dioxide, on aquatic organisms, including the potential for endocrine disruption, photo-activation, and other reported effects on molecular, cellular, organismal, population, community-level, and ecosystem-level endpoints. This report also focused on identifying any potential Endangered Species Act listed organisms or those that are the subject of targeted management plans that have a likelihood of exposure. A summary of the committee's findings, conclusions, and knowledge gaps related to effects from inorganic UV filters in addition is included in this presentation.

**5.09.T-06 Implications of Potential Changes in Sunscreen Usage on Public Health**
***Karen Glanz****, University of Pennsylvania*
An ad hoc committee of the National Academies of Sciences, Engineering, and Medicine on the Environmental Impact of Currently Marketed Sunscreens and Potential Human Impacts of Changes in Sunscreen Usage reviewed the state of the science on the use of the active ingredients in sunscreens (UV filters) currently marketed in the United States. As part of their report, the committee discussed the potential for changes in sunscreen usage and the implications of these changes on human health. The report summarized the literature regarding the use of sunscreen to prevent skin damage in humans from excess exposure to ultraviolet radiation from sunlight. Additionally, it reviewed what is known about sunscreen use patterns for aquatic and other outdoor activities and the potential for changing usage due to environmental concerns. The anticipated health consequences of reduced use of currently marketed sunscreen ingredients or substitution of alternative UV filters is discussed. A summary of the committee's findings, conclusions, and knowledge gaps related to the human health implications of changing sunscreen usage is included in this presentation.

**5.09.T-07 Discussion of the Report by the National Academies Committee on Environmental Impact of Currently Marketed Sunscreens and Potential Human Impacts of Changes in Sunscreen Usage (1 of 2)**

***Lawrence Barnthouse*[1]**, *Scott Belanger[2], Karen Glanz[3], Christopher Higgins[4], Rebecca Klaper[5], Charlie Menzie[6], Carys Louise Mitchelmore[7] and Emily Twigg[8], (1) LWB Environmental Services, (2) Procter & Gamble Company (retired), (3) University of Pennsylvania, (4) Colorado School of Mines, (5) University of Wisconsin, Milwaukee, (6) Exponent, (7) University of Maryland Center for Environmental Science, (8) National Academies of Sciences, Engineering, and Medicine*

This half hour discussion session will be comprised of a moderated panel of members of the National Academies Committee on Environmental Impact of Currently Marketed Sunscreens and Potential Human Impacts of Changes in Sunscreen Usage. Included as part of the discussion will be perspectives on the multidisciplinary and multipartite approach taken by the committee and by the guest experts consulted by the committee as part of its information gathering. As the issue has garnered substantial interest from all segments of the SETAC community, state and national interests, academia, and industry, questions and comments from the audience will be invited. The discussion session will also include a very brief overview of the conclusions and recommendations of the report. A 4-page summary of the report will be available as background for attendees and also available upon request.

**5.09.T-08 Discussion of the Report by the National Academies Committee on Environmental Impact of Currently Marketed Sunscreens and Potential Human Impacts of Changes in Sunscreen Usage (2 of 2)**

***Carys Louise Mitchelmore*[1]**, *Lawrence Barnthouse[2], Scott Belanger[3], Karen Glanz[4], Christopher Higgins[5], Rebecca Klaper[6], Charlie Menzie[7] and Emily Twigg[8], (1) University of Maryland Center for Environmental Science, (2) LWB Environmental Services, (3) Procter & Gamble Company (retired), (4) University of Pennsylvania, (5) Colorado School of Mines, (6) University of Wisconsin, Milwaukee, (7) Exponent, (8) National Academies of Sciences, Engineering, and Medicine*

This half hour discussion session will be comprised of a moderated panel of members of the National Academies Committee on Environmental Impact of Currently Marketed Sunscreens and Potential Human Impacts of Changes in Sunscreen Usage. Included as part of the discussion will be perspectives on the multidisciplinary and multipartite approach taken by the committee and by the guest experts consulted by the committee as part of its information gathering. As the issue has garnered substantial interest from all segments of the SETAC community, state and national interests, academia, and industry, questions and comments from the audience will be invited. The discussion session will also include a very brief overview of the conclusions and recommendations of the report. A 4-page summary of the report will be available as background for attendees and also available upon request.

**5.09.P Environmental Impact of Currently Marketed Sunscreens and Potential Human Impacts of Changes in Sunscreen Usage**

**5.09.P-Mo155 Thyroid Hormone Disruption Potentials of Major Organic UV Filters of Octisalate, Homosalate, and Diethylamino Hydrobenzoyl Hexyl Benzoate in Zebrafish (Danio rerio) and Rat Pituitary (GH3) Cells**

***Ah-Reum Jo***, *Ba Reum Kwon, Eun-Jin Kim, Yeonju Nam, Gowoon Lee and Kyungho Choi, Seoul National University, Korea, Republic of (South)*

Octisalate (OS), homosalate (HS), and diethylamino hydroxybenzoyl hexyl benzoate (DHHB) have been widely used as organic ultraviolet (UV) filters in many personal care products. These chemicals are often used in combination to enhance to protect against the deleterious effects of UV. Despite their heavy usage, the knowledge of their endocrine disruption, especially thyroid hormone, is limited. This study was conducted to investigate the thyroid disruption potentials of OS, HS, or DHHB using zebrafish (*D. rerio*) and rat pituitary (GH3) cells. In addition, we evaluated the mixture effects of OS and HS based on their thyroid disrupting mode of action identified from the zebrafish *in vivo* experiment. In embryo-larval zebrafish, thyroid hormones (THs;

TT4, TT3, FT4, FT3) and thyroid-stimulating hormone (TSH) were measured following 120 hours of exposure. Transcriptional changes of TH regulating genes were quantified in zebrafish. For GH3 cells, genes belonging to the central regulation of THs were analyzed to determine mixture effects after 48 hours of OS+HS exposure. As consequence, in zebrafish larvae, OS and DHHB exposure induced significant changes in all measured THs and TSH ($p$<0.05) but the directions of alteration were different. Exposure to OS significantly increased TH levels, while DHHB significantly decreased TH levels. For HS, an increasing trend of TH levels was observed however, the changes were not statistically significant. OS and DHHB generally up-regulated the genes related to central regulation of THs (*trh*, *trhr*, *tshβ*, *tshr*, *trα*), synthesis (*nis*, *tpo*), and metabolism (*dio1*, *dio2*, *dio3*). Exposure to HS altered gene transcription of TH synthesis- (*tg*), transport- (*ttr*), and metabolism (*dio1*, *ugt1ab*, and *sult1st5*). In GH3 cells, OS, HS, and DHHB significantly up-regulated *trhr* gene. For mixture exposure, both salicylates were mixed based on the NOAELs (no observed effect levels) determined for gene transcription in GH3 cells. OS+HS mixture caused significant up-regulation of *Trhr* gene at ≥NOAEL x1/2 concentrations, suggesting additivity or greater-than-additive effects. Overall, the results of our study demonstrate the potential thyroid disrupting effects of UV filters commonly used in marketed sunscreens. Further studies should be conducted to validate our observation and to understand the mixture effects of chemical exposure *in vivo*.
Acknowledgement: This research was supported by the Korea Ministry of Environment (MOE) Project No. 1485017189

## 5.10 Fluorine-Free Replacements: A Real-World Vignette for Avoiding Regrettable Substitutions

### 5.10.T-01 Introductory Remarks: Fluorine-Free Replacements

***Jamie Suski***[1], *Lindsay Holden*[2], *Andrew East*[2] *and Christopher J. Salice*[3], *(1) EA Engineering, Science, and Technology, Inc., PBC, (2) U.S. Army Public Health Center, (3) Towson University*

Session co-chairs will provide brief remarks on the assessment of alternative products intended to replace fluorine-containing aqueous film forming foam (AFFF). The session is focused on the importance of assessing hazard through a variety of chemical and biological data perspectives. Presentations span biodegradation; toxicity testing in freshwater, marine, avian, and mammalian systems; life cycle assessment; and hazard tradeoffs.

### 5.10.T-02 Testing Biodegradability of Aqueous Film Forming Foam Formulations in an Aerobic Environment

***Mahsa Modiri Gharehveran***[1], *Alexandria Walus*[1], *Todd Anderson*[2], *Seenivasan Subbiah*[2], *Jennifer Guelfo*[2], *Matthew Frigon*[1] *and Jamie Suski*[1], *(1) EA Engineering, Science, and Technology, Inc., PBC, (2) Texas Tech University*

Per- and polyfluoroalkyl substances (PFAS) have been historically used in fire-suppression activities including aqueous film-forming foam (AFFF) given their unique structures and characteristics. However, the same properties that make them durable, resistant, and valuable also make them persistent, bioaccumulative and toxic. This has led to recent investigations on alternative PFAS-free AFFF formulations. Such foams should contain certain classes of chemicals to be effective while being more environmentally friendly. Therefore, it is necessary to thoroughly characterize the constituents, and perform necessary biodegradability and toxicity studies. As part of the Department of Defense's Strategic Environmental Research and Development Program (SERDP), we performed comprehensive constituents' characterization and assessed the biodegradability of seven PFAS-free AFFF and a reference PFAS-based AFFF. We discovered that most of PFAS-free formulations share similar constituents with similar biodegradability through 28-days reaction period. In PFAS-based AFFF, 4:2 and 6:2 fluorotelomer sulfonates were identified with 6:2 FTS concentration at ~ 9000 ppb. In biodegradation studies, the concentrations of 4:2 FTS and 6:2 FTS decreased while generating some perfluoroalkyl acids (PFCAs). These results dramatically increase the current knowledge on the biodegradability of AFFF alternatives but also highlights the need for future efforts on development of novel PFAS-free AFFF formulations that are more biodegradable than PFAS-based AFFF. Overall, the constituent's characterization, the experimental overview, and the results on biodegradability of eight different AFFF formulations including one PFAS-based AFFF will be discussed.

**5.10.T-03 Acute and Chronic Toxicity of Candidate Fluorine-Free AFFF Replacement Formulations to Five Marine Species**

***Ed Wirth***[1]*, Peter B. Key*[1]*, Katy W. Chung*[1]*, Jonathan Stewart*[2] *and Marie E. DeLorenzo*[1]*, (1) National Oceanic and Atmospheric Administration (NOAA), (2) College of Charleston*

Per and polyfluorinated alkylated substances (PFAS) compounds have been used in industrial applications for decades. Detrimental effects to environmental and human health have now been identified and attributed to many of the chemicals classified as PFAS and industry is beginning to target replacement chemicals in order to minimize these newly accepted risks. PFAS have been used as an important component in aqueous film-forming foams (AFFF) used in fire suppression, thus the Department of Defense is seeking toxicity data for fluorine-free AFFF candidate foams. A number of projects are underway to characterize the effects of the potential AFFF replacement foams on non-target species. NOAA is actively determining acute and chronic toxicity thresholds for these selected fluorine-free AFFF formulations in marine and estuarine taxa. This effort is part of a larger collaborative project funded by the Strategic Environmental Research and Development Program (SERDP; ER20-1518) that tasked NOAA, US Army Engineer Research and Development Center (ERDC) and NIST with establishing toxicity thresholds on six replacement AFFF candidates (Avio F3 Green, Bio-Ex Ecopol A, Fomtec Enviro, Solberg Re-Healing, NRL-502w and NFD 20-391) and one actively used PFAS-containing reference AFFF (Buckeye Platinum Plus). Toxicity thresholds are reported based on nominal formulation concentrations to acknowledge the proprietary nature of these AFFFs. Acute 24-96h toxicity test thresholds are reported for eastern mud snail larvae *(Tritia obsloeta*), sheepshead minnow larvae (*Cyprinodon variegatus*), larvae mysid shrimp (*Americamysis bahia*) and the pelagic copepod (*Acartia tonsa*). Results for chronic testing at this time include the inhibition of growth rate in the diatom (*Phaeodactylum tricornutum*). In terms of acute toxicity, larval *T. obsoleta* were generally the most sensitive marine species tested for all AFFFs (48 h LC50 range 1.32-19.9 mg/L). Growth rate inhibition (IC50) for the diatom ranged from 0.15 to 5.18 mg/L. These toxicity threshold results will be used to support the DoDs decisions regarding the environmental specifications used to select appropriate replacement AFFFs.

**5.10.T-04 Acute and Chronic Toxicity of Candidate Fluorine-Free AFFF Replacement Formulations to Five Freshwater Species**

***David W. Moore***[1]*, Guilherme R. Lotufo*[1]*, Alan Kennedy*[1]*, Lauren May*[1]*, Ashley Harmon*[2]*, Tom Biber*[2] *and Nicolas Melby*[1]*, (1) U. S. Army Corps of Engineers, (2) U.S. Army Engineer Research and Development Center*

The Department of Defense (DoD) is actively engaged in research to identify suitable replacements in response to human health and environmental concerns arising from the historic use of aqueous film-forming foam (AFFF) formulations containing per and polyfluorinated alkyl substances (PFAS). The Strategic Environmental Research and Development Program (SERDP) is currently supporting several efforts to determine environmental bioavailability and toxicity of selected candidate replacement formulations in relation to traditional PFAS containing AFFF. The work presented here was conducted as a component of a larger effort in collaboration with NOAA and NIST and is focused on establishing acute and chronic toxicity of six candidate replacement formulations in relation to a C-6 PFAS based formulation (Buckeye Platinum Plus). Formulations under evaluation include 4 commercially available foams (Avio F3 Green, Bio-Ex Ecopol A, Fomtec Enviro, and Solberg Re-Healing) and 2 additional formulations currently under development (NRL-502W and National Foam NFD). Due to the proprietary nature of the formulations evaluated it was not possible to measure exposure concentrations based on active ingredients therefore toxicity of neat formulations were evaluated on a nominal basis (i.e., mass of material in known volume). Separate non targeted analysis performed by NIST confirmed relative stability of test exposure solutions. Each of the candidate formulations were evaluated in five freshwater species including the benthic insect (*Chironomus dilutus*), an epibenthic crustacean (*Hyalella azteca*), a pelagic zooplanton (*Ceriodaphnia dubia*) and two pelagic fish species

(*Pimephales promelas* and *O. mykiss*). Nominal acute LC50 values ranged from 8.7 mg/L in 48 hour test with *C. dubia* (Avio F3 Green) to >100 mg/L multiple species (Buckeye Platinum Plus, NRL 502W). *C. dubia* was generally the most sensitive of the species tested. 7-day chronic reproductive tests with *C. dubia* resulted in 25% effect concentrations ranging from 2.6 mg/L (Avio F3) to 45.6 mg/L (NRL 502W). Results of acute and chronic toxicity tests will be presented. Toxicity data derived from this project will be used to support selection of candidate replacement formulations meeting current DoD performance requirements.

**5.10.T-05 Chronic Reproductive Toxicity of Fluorine-Free Firefighting Foams to Northern Bobwhite Quail (Colinus virginianus)**
***Farzana Hossain*[1]**, *Anna Sophia Longwell[1], Nicole M. Dennis[1], Seenivasan Subbiah[1], Adcharee Karnjanapiboonwong[1], Jamie Suski[2] and Todd Anderson[1], (1) Texas Tech University, (2) EA Engineering, Science, and Technology, Inc., PBC*

The development of fluorine-free firefighting foams has been proposed as a way to reduce the potential negative impacts of foams containing per- and poly-fluoroalkyl substances (PFAS). The previous use of long-chain PFAS compounds in firefighting foams has led to issues with persistence, bioaccumulation, and toxicity. While there are potentially fewer issues with these new fluorine-free foams in terms of persistence, it is prudent to also evaluate the potential ecotoxicity of these fluorine-free foam products given the overall lack of ecotox data. An acute oral toxicity test with six of these fluorine-free foams (and a PFAS-containing foam for comparison purposes) in Northern Bobwhite quail (*Colinus virginianus*) indicated LD50 values at approximately 1500 mg/kg body weight. Subsequent oral chronic exposure studies on adult pairs of Northern Bobwhite quail through drinking water were conducted with these foams: Buckeye Platinum Plus C6, National Foam Avio Green KHC, National Foam NFD 20-391, and Solberg Re-Healing Foam at three different exposure concentrations; 0.01%, 0.1% and 0.25%. Adults were monitored for survival, growth, and reproductive output. Hatching success, chick survival, and 21-d growth were also determined. Adult survival from chronic oral exposure of two fluorine-free foams, National Foam NFD 20-391 and Solberg Re-Healing Foam was 100%. Average daily intake (ADI) for the Solberg Re-Healing Foam based on water consumption was 16.1, 149 and 360 mg/kg body weight/day at exposure concentrations of 0.01%, 0.1%, and 0.25%, respectively. ADI for the National Foam NFD 20-391 was 16.6, 137, and 406 mg/kg body weight/day at exposure concentrations of 0.01%, 0.1%, and 0.25%, respectively. There were no statistically significant treatment effects observed on adult body weight change ($p = 0.09$). No significant treatment effect was observed for eggs laid by adults among treatment groups in comparison to control. Chronic toxicity of the foams will be assessed based on ADI, reproductive, and health effects data in quail to derive toxicity reference values (TRVs).

**5.10.T-06 Toxicity Assessment of Alternative Aqueous Film Forming Foams**
*Lindsay Holden, **Andrew East**, Allison Narizzano and Michael Quinn, U.S. Army Public Health Center*
Current aqueous film forming foams (AFFFs) used by the Department of Defense (DoD) contain per- and polyfluoroalkyl substances (PFAS). The extent of the toxicological impact of PFAS exposure is an active area of research, but there is growing evidence of negative health impacts across multiple biological systems.Research, development, test, and evaluation (RDT&E) to develop and field alternative AFFFs that are less hazardous to human health and the environment is vital to the readiness of the DoD. Moreover, there is signification potential for hazard and cost reduction if a tiered-testing approach to toxicity hazard determination is incorporated throughout the RTD&E pipeline. The Strategic Environmental Research and Development Program (SERDP) is supporting extensive toxicity tests with six candidate PFAS-free AFFFs and one PFAS-containing AFFF as a comparator. This toxicity assessment compiles data from available literature, quantitative structure-activity response modeling, and data from studies performed by multiple labs and ranks the likelihood of hazard across the seven AFFFs. Generally, the PFAS-free AFFF products appear to have lower likelihood of environmental persistence and bioaccumulation, and have lower oral human health toxicity as compared with products containing PFAS. Most products in their concentrated form may cause dermal and ocular irritation and

may be hazards to firefighters without the use of personal protective equipment. Oral exposure to relatively high concentrations may cause gastrointestinal distress and one product has the potential for anemia and liver injury at high concentrations (approximately 20% dilution). Aquatic toxicity may be a concern from direct or repeated environmental releases, especially for organisms that live at or near the water/air interface where foam may collect and concentrations may elevate. Aquatic organisms appear more sensitive to exposures than mammalian species and there is still a large gap in knowledge regarding the unidentified product components by the manufacturers. Each product contains chemical constituents that are below the legal reporting threshold and/or are protected as proprietary business information and are therefore not disclosed in safety data sheets, which remains a barrier to quantify hazards related to potential discharges or disposal.

**5.10.T-07 Discussion - Fluorine-Free Replacements**

***Lindsay Holden***[1]*, Andrew East*[1]*, Jamie Suski*[2] *and Christopher J. Salice*[3]*, (1) U.S. Army Public Health Center, (2) EA Engineering, Science, and Technology, Inc., PBC, (3) Towson University*

**5.10.T-08 Fit-For-Purpose Performance: Evaluating Alternatives to Aqueous Film Forming Foam (AFFF)**

***Monika A. Roy***[1]*, Pam Eliason*[2]*, Joel Tickner*[1]*, Elizabeth Harriman*[2]*, Joy Onasch*[2] *and Molly Jacobs LeFevre*[1]*, (1) University of Massachusetts, Lowell, (2) Toxics Use Reduction Institute*

The U.S. Department of Defense (DoD) is under a Congressional mandate to phase out use of PFAS-based Aqueous Film Forming Foam (AFFF) by 2024. For defense applications, performance is a primary consideration in making decisions regarding "mission critical" substitutes, alongside hazard/risk and cost. Regrettable substitutions can occur if specific performance metrics cannot be achieved under real-world conditions. However, performance can also be a barrier to the adoption of safer and available substitutes if evaluations are based around a single "best in class" performance metric, require performance through specific mechanisms, or require consideration of adoption feasibility as part of the performance evaluation. "Fit-for-purpose" performance is a strategy for assessing performance based on application-specific contexts with the key goal of minimizing extended debates over whether alternatives perform equally to the incumbent chemistry. A fit-for-purpose performance approach underscores the importance of evaluating whether the function of the chemical, material, product, or process of concern achieves sufficient performance for the application, expands the thinking around performance as a range, and acknowledges important considerations around tradeoffs with environmental health and safety. This approach was developed into a general guidance as part of a DoD sponsored project to improve the use of alternatives assessment in evaluating alternatives for military land-based applications of AFFF. Here, we share a high-level application of this guidance for thinking through fit-for-purpose performance for AFFF alternatives, which was built from interviews conducted with firefighting experts. The goal of this work is to facilitate the transition to safer and effective options to meet identified functional needs, while avoiding "paralysis by analysis" and the use of overly prescriptive standards that prevent the adoption of safer substitutes for specific use conditions. Ultimately, potential tradeoffs between performance, toxicity, and cost need to be transparently discussed during substitution decisions. Where options do not currently exist that can meet environmental, technical, and economic performance requirements, investments in R&D in new options will be required.

**5.10.P Fluorine-Free Replacements: A Real-World Vignette for Avoiding Regrettable Substitutions**

**5.10.P-Tu136 An Evaluation of the Chronic Oral Toxicity of Fomtec Enviro USP, a Fluorine-Free Firefighting Foam, to Northern Bobwhite Quail (Colinus virginianus)**

***Anna Sophia Longwell***[1]*, Farzana Hossain*[1]*, Seenivasan Subbiah*[1]*, Adcharee Karnjanapiboonwong*[1]*, Jamie Suski*[2] *and Todd Anderson*[1]*, (1)Texas Tech University, (2) EA Engineering, Science, and Technology, Inc., PBC*

Long chain per- and poly-fluoroalkyl substances (PFAS) have been the standard active chemicals used in aqueous film forming foams (AFFF) since the mid-1960s. Some characteristics of PFAS are environmental persistence, ability to resist degradation at high temperatures, bioaccumulation, and the ability to travel long distances from the point of release. As an alternative, non-fluorinated firefighting foams are being introduced with the intent of having a decreased ecological and environmental impact. In a chronic study, Northern Bobwhite quail (Colinus virginianus) were used to test the ecotoxicity of one candidate foam. Fomtec Enviro USP is a fluorine-free commercial AFFF used primarily for extinguishing class B hydrocarbon fuel fires. Following a photo-stimulation phase, breeding pairs of quail were exposed over 60 days to 0.01%, 0.1%, and 0.25% Fomtec in drinking water. Endpoints of the study included survival, growth, and reproductive output. Water consumption was used to determine average daily intake (14.2, 156, and 394 mg/kg/day for the 0.01%, 0.1%, and 0.25% exposures, respectively). Over the 60 days, control females laid an average of 59 eggs compared to 28 (0.01% exposure), 51 (0.1% exposure), and 56 (0.25% exposure). Egg hatching success and chick survival following adult oral exposure in drinking water to Fomtec Enviro USP was negatively impacted compared to controls. Our data on chronic toxicity in this avian model helps to fill data gaps for these non-fluorinated foam products, many of which have little toxicological information.

**5.10.P-Tu137 Acute Toxicity Response of the Eastern Oyster, Crassostrea virginica, After Exposure to Per- And Polyfluoroalkyl Substance-Free Aqueous Film-Forming Foams**

***Jonathan Stewart***[1]*, Katy W. Chung*[2]*, Peter B. Key*[2] *and Ed Wirth*[2]*, (1) College of Charleston, (2) National Oceanic and Atmospheric Administration (NOAA)*

Aqueous film forming foams (AFFFs) are an important tool for fire suppression used in the aviation and defense industries. These foams have historically contained per- and polyfluoroalkyl substances (PFAS) which are now recognized as environmental toxicants with known impacts on biological systems. In an effort to reduce PFAS impacts, current AFFFs are being replaced with PFAS-free formulations. The objective of this research is to evaluate six selected PFAS-free AFFFs relative to a currently used PFAS-containing AFFF. The Eastern Oyster (*Crassostrea virginica*) has been used as a benchmark test organism for environmental and chemical toxicity testing. In this study, the AFFF impacts on acute larval survival was measured over a 48-h exposure and these calculated acute larval toxicity thresholds (LC50) suggest a majority of the tested formulations would be considered “Slightly Toxic” according to US EPA toxicity definitions. To date, significant larval mortality was observed at a 100 mg/L dose for three of the PFAS-free AFFFs. Additional testing will evaluate effects on oyster settlement and shell deposition. These data complement a larger effort assessing potential PFAS-free AFFF hazards as decisions are made to reduce PFAS-containing AFFF use.

**5.10.P-Tu138 An Evaluation of Aquatic Receptor Sensitivities to Novel Fluorine-Free Firefighting Foam Versus Short Chain Per- and Polyfluoroalkyl Substances Aqueous Film Forming Foam Products**

***Shelly Hudson****, Jamie Suski, Michael Chanov, Sara Lanasa and Taylor Pearson, EA Engineering, Science, and Technology, Inc., PBC*

Per- and Polyfluoroalkyl substances (PFAS) have surged to the forefront of environmental research due to their persistence in the environment and wide-spread use of PFAS-containing aqueous film forming foam (AFFF) in industrial and commercial practices as well as by the Department of Defense (DoD) for fire suppression needs. Candidate AFFF-alternatives [fluorine-free (FF) foam] products and new generation short-chain PFAS AFFF are emerging to address the gap created by the phasing out of PFAS AFFF. In turn, there is an imperative need to characterize environmental concerns of FF foam products. This research aims to fill ecotoxicology data gaps for several ecological receptors, including algae, macroinvertebrates, fish, birds, and reptiles, and to provide an assessment of the biodegradation potential of FF foams and the new generation short-chain PFAS AFFF currently in use. Collectively, these data will inform decisions regarding potential environmental concerns associated with replacement firefighting foams to prevent further compounding the global concern of prior legacy AFFF application. This presentation focuses on the aquatic toxicity testing portion of the project. Acute toxicity tests were performed on green algae (*Raphidocelis subcapitata*), invertebrates (Midge, *Chironomus tentans*), and fish (Fathead Minnow, *Pimephales promelas*). Definitive acute toxicity data (LC50) for the FF products for algal toxicity are 0.69 – 259.59 mg/L and the comparative short-chain PFAS AFFF was near the mid-point within this range (136.08 mg/L). The definitive acute toxicity data (LC50) for the FF products for chironomids are 24.51 – 1,036.8 mg/L and the comparative short-chain PFAS AFFF was near the higher end of this range in toxicity (885.2 mg/L). Finally, definitive acute toxicity data (LC50) for the FF products for fathead minnows are 2.31 – 813.12 mg/L with the short-chain PFAS AFFF serving as the high end of this range. Applying the alternatives assessment criteria developed by the U.S. Environmental Protection Agency, two of the seven PFAS-free foams tested appear to fall within moderate-very high hazard for all three test species. This appears to be toxicity specific and not an indirect effect related to the chemical oxygen demand of surfactants within the foams. These data are undergoing further synthesis and comparison while definitive chronic toxicity testing is expected to be conducted in the near term.

**5.10.P-Tu139 Assessing the Ecotoxicity of Fluorine-Free foams to House Crickets (Acheta domesticus) via a Novel Exposure System**

***Taylor S. Anderson** and Christopher J. Salice, Towson University*

Per- and polyfluoroalkyl substances (PFAS) represent a large class of manufactured, persistent chemicals that have been used in a variety of commercial and industrial applications due to their physical and chemical properties. Use of PFAS in aqueous film forming foams (AFFFs) has been particularly prominent and their widespread usage has led to soil, groundwater and surface water contamination, and presents concern for ecological and human health impacts. Consequently, there is a need to develop alternative fluorine-free fire-fighting products that provide the same effectiveness as PFAS-based, without harmful ecological effects. The goal of this research was to assess the toxicity of fluorine-free alternatives using a novel drinking-water exposure method, the "Falcon Dress", on a model terrestrial invertebrate. House crickets (*Acheta domesticus*) were used as the model invertebrate due to their abundance in nature, relative ease of maintenance, availability, and significance in the food chain, particularly for reptilian and avian species. Two, 120-hour screening studies utilizing the Falcon Dress method were used to determine toxicity. Crickets were exposed to four experimental solutions and one control solution in each study. Controls were water only. Seven total fluorine-free solutions at 3% concentration were tested: Angus Fire, Buckeye, NRL 502W, FOMTEC, National Foam, BIOEX, SOLBERG. Buckeye was included as a reference control formulation because it is a C6 fluorinated AFFF and was assessed twice because of observed toxicity in the first study. Both screening studies yielded 0% mortality for all controls. Buckeye yielded an average of 30% and 42.5% mortality on day 5 of the first and second study, respectively. In the first screening study, Angus fire yielded an average mortality of 12.5%, NRL 502 was 5%, and FOMTEC was 2.5%. In the second screening study, National foam yielded an average mortality of 22.5%, BIOEX was 15%, and SOLBERG was 5%. These results give insight into the possible toxic effects of the fluorine-free replacement chemicals and, preliminarily, indicate lower toxicity than Buckeye. Utilizing toxicity assessments can help determine what chemicals are appropriate for replacing PFAS, without creating more or new harmful ecological impacts.

**5.10.P-Tu140 Acute and Reproduction Toxicities of Fluorine-Free Aqueous Fire-Fighting Formulations for Soil Invertebrates.**

***Roman Kuperman**[1], Michael Simini[1], Guilherme R. Lotufo[2] and Robert Boyd[3], (1) U.S. Army DEVCOM Chemical Biological Center, (2) U. S. Army Corps of Engineers, (3) U.S. Army Engineer Research and Development Center*

Legacy aqueous film-forming foam (AFFF) formulations containing perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) have been linked to accumulation in soil invertebrates and terrestrial plants in previous studies with the potential for biomagnification in terrestrial food-webs. Alternative fluorine-free surfactant formulations are being developed and evaluated for their ability to meet current Department of Defense (DoD) performance requirements. The relative toxicities of fluorine-free AFFF-alternatives (FF-AFFF), as compared with legacy AFFF formulations are not known. We have developed ecotoxicological data for seven candidate FF-AFFF formulations and a legacy AFFF formulation by determining chronic toxicity for soil invertebrates collembolan *Folsomia candida,* earthworm *Eisenia andrei,* and potworm *Enchytraeus* *crypticus*. Test species were exposed in separate studies to each formulation in a natural soil, Sassafras sandy loam, which has characteristics (low clay and organic matter content) expected to support high bioavailability for these materials. Toxicity data derived from this project will be used to develop Soil Ecotoxicological Risk Factors (SERF) to assess which FF-AFFF formulations would exhibit lesser environmental toxicity, while meeting the current DoD performance requirements.

**5.10.P-Tu141 Defining Criteria for a "Safer" Non-Fluorinated AFFF alternatives: Guidance to Strengthen Future Alternatives Assessments**

***Molly Jacobs LeFevre*** *and Joel Tickner, University of Massachusetts, Lowell*

Policies in the United States and beyond are driving efforts to phase out the use of Aqueous Film Forming Foam (AFFF) products used to extinguish flammable liquid fires. AFFF contains per- and polyfluorinated alkyl substances (PFAS), which are highly persistent, bioaccumulative and toxic substances. To support alternative assessments of PFAS-free alternatives to AFFF, this project developed specific criteria to support determining whether alternatives are safer as compared to current PFAS-containing AFFF products. The criteria should be considered the *minimum requirements* for a safer AFFF alternative determination. Criteria are drawn from existing approaches, in particular the Organization for Economic Cooperation and Development's (OECD) "Guidance on Key Considerations for the Identification and Selection of Safer Alternatives" and supplemented by others such as the Green Screen Certified™ for Fire Fighting Foam and the US Environmental Protection Agency's Safer Choice criteria. This presentation will review the minimum requirements for a safer AFFF alternative determination, which includes a Part A and Part B. Conformance with Part A ensures that problematic groups of substances are not used as ingredients (intentionally added or impurities/residuals) in an alternative formulation for such formulation to be considered "safer". Conformance with Part B includes ten hazard endpoints and/or associated hazard classifications that cannot be of "high" concern – based on applying criteria/thresholds using the Globally Harmonized System (GHS) of Classification and Labelling of Chemicals – for an alternative AFFF formulation to be considered safer. Going beyond the minimum is recommended *wherever and whenever possible* to further reduce the likelihood that an alternative to AFFF will result in unintended consequences to the environment, workers, and the public. As such, the guidance outlines additional hazard endpoints to consider beyond the minimum requirements.

**5.11 In silico NAMs: Recent Developments and Regulatory Applications**

**5.11.T-01 Comparisons of Biodegradation and Aquatic Toxicity Machine Learning Models**

*Thomas Lane, Fabio Urbina and* ***Sean Ekins****, Collaborations Pharmaceuticals, Inc.*

Understanding the environmental fate and potential toxicity of large numbers of molecules is an important regulatory need across industries. There are now many public datasets and machine learning algorithms that can be applied and used to identify the best model or generate consensus approaches. We have focused on models for biodegradation and aquatic toxicity. For biodegradation we curated data from seven sources including databases and publications. The final dataset contained 3428 unique compounds with 962 classified as "readily biodegradable". For aquatic toxicity we built models for fish, daphnia and algae using the EPA ECOTOX and the Ecotoxicity Test Database from the Ministry of the Environment of Japan and publications. Two datasets were built representing high and low/no toxicity (≤1mg/L and ≥100mg/L). For fish the final dataset (2821 compounds) the high and low/no toxicity datasets had 818 and 624 actives, respectively. For daphnia the dataset (1377) the high and low/no toxicity datasets having 345 and 484 actives, respectively. For the algae dataset (1129) the high and low/no toxicity datasets had 389 and 126 actives, respectively. Models were built using 8 machine learning algorithms and the predictive nature assessed using 5-fold nested cross validation for all but deep learning (prediction of a single 20% leave-out set). The best biodegradation model was generated with XG-Boost (ROC 0.88, Accuracy 0.84). The models for aquatic toxicity indicated that in each case different algorithms performed the best. These models will now be incorporated in MegaTox and used for scoring libraries of molecules to prioritize testing.

**5.11.T-02 Modeling Freshwater Mussels to Assess Risks to Threatened and Endangered Species**

***Maxime Vaugeois***[1], *Adrian Moore*[2], *Valery Forbes*[2], *Richard Brain*[1], *Daniel Hornbach*[3] *and Nika Galic*[1], *(1) Syngenta Crop Protection, (2) University of Minnesota, (3) Macalester College*

Freshwater mussels are a highly diverse group of animals playing essential roles in freshwater ecosystems. They are also one of the most endangered taxonomic groups in North America: 70% of the 296 species currently identified in the US are considered threatened, endangered, or of special concern. Many factors are identified as potential drivers of decline, such as physical habitat degradation, invasive species, complex life cycle (larvae require a host to develop into independent juveniles), and chemical pollution. However, because of the prohibition on testing of endangered species and the difficulty of working with freshwater mussels in the laboratory, it is unclear how exposure to various stressors translates into long-term fitness consequences for distinct species with specific physiology and life history. Consequently, it is important to develop predictive methods to better assess the risks posed to threatened and endangered freshwater mussels by a multitude of stressors.

We developed Dynamic Energy Budget (DEB) models for 47 species belonging to three life-history categories (opportunistic, periodic, and equilibrium) defined in a previous study. DEB theory describes how physiological processes and life-history properties are linked to underlying bioenergetics. It is based on generic rules, which allows for broad applicability across species with the same mathematical formalism. DEB models are increasingly applied to quantify the physiological responses of individuals to stressors in their environment. We compared how exposure to stress of identical intensity impacted growth, reproduction, and development across species and life-history categories. We found that impacts on development (age-at-puberty) and reproduction (egg production) were more variable among species than impacts on growth. We also found that the intensity of simulated impacts was driven more by species-specific energetics than life-history categorization.

Because long-term population viability of these listed species is the focus of risk assessment (specifically in Step 3), we plan to integrate the developed DEB models into agent-based models to evaluate how perturbations to listed mussel bioenergetics determine population-level outcomes. Ultimately, our approach will generate insights about the sensitivity of physiological processes, life-history traits, and populations from exposure to various stressors, including pesticides, of data-sparse threatened and endangered species.

**5.11.T-03 Modeling pesticide effects on multiple threatened and endangered Cyprinid fish species to support decision making.**

***Chiara Accolla***[1], *Amelie Schmolke*[1], *Andrew Jacobson*[1], *Colleen Roy*[1], *Valery Forbes*[2], *Richard Brain*[3] *and Nika Galic*[3], *(1) Waterborne Environmental, Inc., (2) University of Minnesota, (3) Syngenta Crop Protection*

Mechanistic models are invaluable in ecological risk assessment (ERA) because they facilitate extrapolation of organism-level effects to population-level effects while accounting for species life history, ecology, and vulnerability. Therefore, models are particularly useful for assessing potential risks of pesticides to threatened and endangered species, for which data collection and laboratory tests are challenging or impossible. We developed a model framework to compare the potential effects of the fungicide chlorothalonil across several listed species of cyprinid fish and explored species-specific traits of importance at the population level. The model is an agent-based model based on the dynamic energy budget (DEB) theory. As a case study, we considered four listed species of Cyprinidae: Humpback chub (*Gila cypha*), Spikedace (*Meda fulgida*), Topeka shiner (*Notropis topeka*), and Devils River minnow (*Dionda diaboli*). Potential direct lethal and/or sublethal effects on individual fish were considered as well as indirect effects through reduction in prey. We calibrated four effect sub-models to account for different effect pathways based on experimental data from exposure to chlorothalonil. Toxicokinetic-toxicodynamic models were used for representing direct effects, whereas indirect effects were described by decreasing food availability. Exposure profiles were constructed based on a degradate (hydroxychlorothalonil), given the relatively short half-life of the parent chlorothalonil. We performed two kinds of simulations (i) we applied all effect sub-models simultaneously and considered different exposure magnification factors (EMFs); (ii) we sequentially added the different effect sub-models to test their relative importance. We demonstrated that exposure affected population dynamics depending on species-specific life-history traits and processes (i.e., density dependence). Different EMFs were required to achieve a comparable population decrease across species. Moreover, sequentially adding effect sub-models resulted in different outcomes depending on the interplay of life-history traits and density-dependent compensation effects. We conclude by stressing the importance of using models in ERA to account for species-specific characteristics and ecology, especially when dealing with listed species and in accordance with the necessity of reducing animal testing.

**5.11.T-04 A Framework for Evaluating the Need for Ecotoxicity Testing**

***Paul C. DeLeo**, American Chemistry Council*

Over the past two years, the U.S. Environmental Protection Agency (EPA) Office of Pollution Prevention and Toxics (OPPT) has exercised new authority to require chemical testing under the Toxic Substances Control Act for the first time since it was reformed in June 2016. Among the tests ordered by EPA were ecotoxicity tests for soil-dwelling, sediment-dwelling and avian species. The recent (April 2022) test orders were the first where vertebrate animal testing was required since TSCA reform. EPA requested Avian Dietary Toxicity Testing (OCSPP 850.2200) and/or Avian Reproduction Test (OCSPP 850.2300) for three high priority substances for risk evaluation under TSCA. The EPA Office of Pesticide Programs, a parallel office to OPPT, has several guidance documents for pesticide registrants to request a testing waiver based on its decades of experience with such data and their value in decision-making. However, OPPT having only recently developed its process for risk evaluation of existing chemicals has not developed or implemented similar guidance. Using lessons learned from the OPP guidance documents for ecotoxicity testing, a tiered approach was developed to evaluate information regarding exposure potential and relative ecotoxicity. Fugacity and multimedia fate models were applied to characterize potential environmental exposure, specifically, the likelihood that a chemical would occur in the environment, in this case, the terrestrial food chain. In addition, results from several models that characterize toxic mode of action (MOA) of a chemical were compared to those MOAs for pesticides that were part of the cohort of substances used to develop the waiver process for sub-acute avian dietary testing. Results are provided for the analysis of the three chemicals for which avian ecotoxicity testing was requested as a case study of the application of this framework.

**5.11.T-05 Exploring the Prospects for Applying NAMs for the Risk Assessment of PFAS**

***James M. Armitage**[1], Alessandro Sangion[2] and Jon A. Arnot[3], (1) AES Armitage Environmental Sciences, Inc, Canada, (2) University of Toronto Scarborough, Canada, (3) ARC Arnot Research & Consulting Inc., Canada*

Per- and polyfluorinated alkyl substances (PFAS) include a diverse and increasing number of chemicals that are of great interest to the public, scientists and regulatory community. Various initiatives to reduce animal testing will require a shift towards greater reliance on New Approach Methodologies (NAMs) for regulatory decisions. The main objective of this presentation is to explore the prospects for applying NAMs for the risk assessment of PFAS, specifically the calculation of Administered Equivalent Doses (AEDs, mg/kg/d) from in vitro bioactivity data. AEDs are typically calculated as the ratio of an in vitro point of departure (e.g., nominal AC50s) and the predicted steady-state plasma or blood concentration ($C_{SS}$) corresponding to an oral dose of 1 mg/kg/d, e.g., AED = AC50/$C_{SS}$ · 1 mg/kg/d. The use of nominal AC50s can be problematic because in vitro disposition is not accounted for whereas predicted $C_{SS}$ can be sensitive to model selection and parameterization. The accuracy of the predicted $C_{SS}$ is also an important consideration. Using compounds selected from the OECD New Comprehensive Global Database (n = 4729 PFAS) as case studies, we applied a mass balance model to simulate in vitro disposition and several established high-throughput 1-compartment toxicokinetic (1Co-TK) models to predict $C_{SS}$. Empirical TK data such as total elimination half-life ($HL_T$) and renal clearance were also compiled for the relatively few PFAS with such measurements. These analyses indicate that there could be some challenges for estimating reliable AEDs for many PFAS. For example, volatilization out of in vitro test systems is probable for approximately 50% of the neutral PFAS simulated meaning that nominal medium concentrations are unreliable as PODs. There are also substantial uncertainties in the predicted in vitro disposition of the well-studied perfluoroalkyl acids (PFAAs). The performance of the selected high-throughput 1Co-TK models for PFAAs was biased towards overestimation of total elimination due to the inability to accurately simulate renal clearance. For other PFAS, obtaining accurate estimates of biotransformation may be difficult. For PFAS with empirical TK data, challenges related to the prediction of $C_{SS}$ can be circumvented and only issues related to in vitro disposition remain. Depending on the PFAS being considered, additional partitioning and TK data may be required to derive AEDs acceptable for risk assessment.

**5.11.T-06 New Approach Methodologies for Modeling Mixtures Toxicity**

***David E. Hines***[1], *Dave Allen*[1] *and Nicole Kleinstreuer*[2], *(1) Inotiv, Inc., (2) NICEATM*

Characterizing risk from exposure to chemical mixtures is challenging because individual ingredients may be present in different concentrations, possess different pharmacokinetic properties, have different bioactivity, and/or interact with each other to alter these properties. In vivo animal experimentation allows testing of whole products, but ethical concerns and practical limitations make it difficult for these experiments to support risk assessment across a wide breadth of mixtures and formulations. Ingredient-based in vitro and in silico techniques are appealing new approach methodologies (NAMs) for this task because data for a single chemical can be used to inform risk assessment for any mixture or formulation containing it. However, applications of these approaches are limited by challenges regarding integration of ingredient data and quantification of mixtures effects. This work develops a novel computational method for ingredient-based mixtures analysis that avoids assumptions about pharmacokinetics and bioactivity commonly applied in existing ingredient-based NAMs. We used a hypothetical mixture and assay system to develop and demonstrate our approach. Specifically, ingredient kinetics were modeled using the httk R package to predict plasma concentrations, which were used in conjunction with concentration-response curves for an inhibition assay to dynamically predict the effect of each chemical. Ingredient effects were integrated to obtain a mixture bioactivity profile for conducting in vitro to in vivo extrapolation (IVIVE). IVIVE results for the mixture using the novel approach were compared to results from an existing ingredient-based NAM that includes assumptions that are typically considered to be conservative and utilizes toxic equivalence factors. Our approach results were more conservative (27.8% lower mixture equivalent administered dose for toxicity) than the toxic equivalence factor NAM, suggesting that understanding non-linear concentration-response relationships is vital for ingredient-based mixtures risk assessment. Furthermore, our approach facilitated dynamic analysis of mixture bioactivity, which is not included in the toxic equivalence factor NAM. Our results demonstrate how NAMs can improve prediction of mixtures bioactivity for targeted biological mechanisms. This project was funded by the National Institute of Environmental Health Sciences, National Institutes of Health (Contract No. HHSN273201500010C).

**5.11.T-07 When Molecules Fall Apart: New Approach Methodologies for Chemicals That Dissociate**

***Jessie Kneeland***, *Chase Butler, Anya Sita Chinniah and Patricia Clyde, Gradient*

To fulfill regulatory requirements and efficiently evaluate chemical safety, it is often important to assess the environmental fate and aquatic toxicity of chemicals while minimizing new testing. Stakeholders are increasingly considering new approach methodologies (NAMs) such as read-across, chemical grouping, and models such as quantitative structure activity relationships (QSARs) to fill data gaps. These approaches and the regulatory guidance for them often focus on neutral organic molecules, but many chemicals of interest contain acidic hydrogens or ions that may dissociate under environmental conditions. This presentation will summarize regulatory guidance and scientific literature on applying NAMS to chemicals that dissociate. Through use of several illustrative examples, we will show how to adapt read-across and modeling approaches for dissociating compounds. We will also highlight some of the limitations to consider. By applying NAMs to chemicals that dissociate, scientists can satisfy regulatory requirements and gain valuable insight on the environmental properties of these chemicals while minimizing costly testing.

**5.11.T-08 In Silico Model-based Exploration of the Relative Importance of Pre- and Post-absorptive Biotransformation in Human Exposure and Toxicokinetic Modeling**

***Shenghong Wang**[1], Li Li[1], Mingliang Fang[2], Zhizhen Zhang[1] and Dingsheng Li[1], (1) University of Nevada, Reno, (2) Nanyang Technological University, Singapore*

Metabolic biotransformation plays an important role in both the absorption and elimination of chemical contaminants: The pre-absorptive intestinal and hepatic biotransformation, also known as the "first-pass effect", lowers the amount of ingested chemical contaminants reaching the systemic circulation, whereas post-absorptive hepatic and intestinal biotransformation reduces the human body burden of chemical contaminants by eliminating them from the systemic circulation. While *in silico* high throughput physiologically based toxicokinetic (PBTK) models consider hepatic biotransformation, intestinal biotransformation is often overlooked, and the relative importance of pre- and post-absorptive biotransformation is unclear. Experimental evidence has shown rapid intestinal biotransformation of certain chemicals, with a half-life at a level of minutes. Neglecting intestinal biotransformation may result in a substantial overestimation of exposure to chemicals. In this presentation, we introduce an *in silico* PBTK model to evaluate (i) the relative importance of pre- and post-absorptive biotransformation in controlling the presence of chemical contaminants in the human body, and (ii) important factors that govern such relative importance. Since *in vivo* biotransformation data are often inadequate for chemical contaminants, the model also includes an *in vitro* to *in vivo* extrapolation module to allow the use of *in vitro* data. The model shows that the relative importance of intestinal biotransformation rates and epithelium permeation greatly impacts intestinal absorption efficiency. In cases of phthalates where biotransformation occurs at a level of minutes, more than 80% of the ingested amount is biotransformed in the gastrointestinal tract before absorption. Overall, our work highlights the importance of intestinal biotransformation in human exposure and toxicokinetic modeling. This model also enables us to more accurately predict the absorption efficiency of chemicals susceptible to intestinal biotransformation.

**5.11.P In silico NAMs: Recent Developments and Regulatory Applications**

**5.11.P-We164 Comparative sensitivity of Daphnia magna and Ceriodaphnia dubia to alcohol ether sulphates**

***Kristin A. Connors** and Jessica Brill, Procter & Gamble*

Regulatory toxicity testing relies on a few dozen model test species and a handful of established test methods/guidelines. These preferences are often based on policy decisions and not necessarily on scientific grounds. For European chemical registrations, the invertebrate *Ceriodaphnia dubia* is not considered a standard test species despite the availability of ISO and US EPA methods and its wide use and acceptance in other countries. A recent metanalysis demonstrated *C. dubia* and *D. magna* were equisensitive in acute and chronic toxicity assays for organic molecules. In this work, the comparative sensitivity of *D. magna* and *C. dubia* to alcohol sulphates (AS) and alcohol ether sulphates (AES) were examined through robust acute toxicity testing of pure chain length AS and AES materials. Historically, AES acute *C. dubia* toxicity has been shown to increase with increasing chain length and decrease with increasing ethoxylates. Here, we show that acute *D. magna* toxicity follows a similar toxicity pattern. Results will be used to construct acute structure-activity relationships for AS and AES to support environmental effects assessment of AES as a mixture. This work was funded in part by a research grant from Environmental Risk Assessment of Surfactants Management (ERASM).

**5.11.P-We165 Leveraging Machine Learning to Predict Species Sensitivity as a Function of Underlying Physiology**

***David A. Dreier**[1], Jochen Zubrod[2], Maxime Vaugeois[1] and Nika Galic[1], (1) Syngenta, (2) Zubrod Environmental Data Science, Germany*

A major challenge in ecological risk assessment is estimating chemical-induced effects across taxa without species-specific testing. This is especially true for species under legal protection such as those listed as threatened or endangered under the Endangered Species Act (ESA), which cannot be taken for ecotoxicological testing. In these instances, surrogate animal models are used to estimate potential effects, but it is currently unclear whether the surrogate data are representative, protective, or realistic. Furthermore, the increasing requirements to assess risk to non-standard species are in stark contrast to the international efforts to reduce animal testing. Where ecotoxicological data may be more challenging to gather, information on species physiology is more available for a broad range of taxa. Physiology is known to drive species sensitivity but understanding about the relative contribution of specific underlying processes is still elusive. Consequently, there remains a need to understand which physiological processes lead to differences in species sensitivity. The objective of our study was to utilize existing knowledge about organismal physiology to both understand and predict differences in species sensitivity. Machine learning models were trained to predict chemical- and species-specific endpoints as a function of both chemical fingerprints/descriptors and physiological variables, including, but not limited to, dynamic energy budget (DEB) properties, life history traits, and potentially taxonomic/phylogenetic relationships. We have explored this approach using publicly available data from the EnviroTox database, with ToxPrints and DEB parameters as predictor variables explaining chemistry and physiology, respectively. We found that a simple random forest model was able to predict chemical- and species-specific endpoints, and that DEB parameters were relatively important in the model. Further work has been conducted to refine and strengthen these models, and to quantify the importance and influence of physiological parameters. We anticipate that our approach will illuminate how physiological parameters drive species sensitivity which will allow more realistic extrapolations of effects across species without the need for additional animal testing.

**5.11.P-We166 Extrapolation of In Vitro Bioactivity Data to Points of Departure (PODs) Using an In Vitro Mass Balance Model (IV-MBM V2.0)**

***James M. Armitage***[1]*, Alessandro Sangion*[2] *and Jon A. Arnot*[3]*, (1) AES Armitage Environmental Sciences, Inc, Canada, (2) University of Toronto Scarborough, Canada, (3) ARC Arnot Research & Consulting Inc., Canada*

In vitro bioactivity and toxicity data are increasingly being generated and considered to facilitate hazard and risk assessment activities. One prominent example is the calculation of Administered Equivalent Doses (AEDs) which can then be compared to traditional in vivo points of departure (PODs) or exposure estimates. AEDs are typically calculated as the ratio of an in vitro POD (e.g., AC50, µM) and the estimated steady-state blood or plasma concentration ($C_{SS}$, µM) corresponding to an oral dose of 1 mg/kg/d (e.g., AED = AC50/$C_{SS}$ · 1 mg/kg/d). A key underlying assumption inherent to the calculation of AEDs following this approach is that the in vitro POD (e.g., AC50) and steady-state blood or plasma concentration are directly comparable exposure metrics (e.g., identical bioavailability in both media). This assumption may be problematic for several reasons stemming from the fact that in vitro PODs are almost always reported on a nominal basis. Because the composition of assay medium can be very different from the composition of plasma and blood and other losses can occur in vitro (e.g., volatilization), there may be substantial “mismatches” between in vitro PODs and $C_{SS}$ with respect to actual exposure. To assess the potential bias in AED calculations that do not explicitly address bioavailability and in vitro disposition, we applied an in vitro mass balance model (IV-MBM v2.0) to a set of neutral and ionizable organics chemicals spanning a wide range of partitioning properties. The model was parameterized to represent in vitro test systems with different exposure conditions particularly with respect to the volume fraction of fetal bovine serum (FBS) added. For semi-volatile hydrophobic chemicals, the difference between the AEDs calculated using nominal AC50s and in vitro PODs estimated using IV-MBM v2.0 can approach two orders of magnitude. There can also be large differences for volatile chemicals due to excessive losses from the in vitro test system. On the other hand, differences between AEDs calculated using nominal AC50s and in vitro PODs estimated using IV-MBM v2.0 are relatively small for more hydrophilic chemicals (i.e., less than a factor of two). The model application demonstrates the importance of considering in vitro disposition and bioavailability issues when extrapolating in vitro bioactivity data to AEDs and then comparing to them to traditional in vivo PODs and exposure estimates.

### 5.11.P-We167 In silico Model-based Exploration of the Applicability of Parallel Artificial Membrane Permeability Assay (PAMPA) to Screen Chemicals of Environmental Concern

***Shenghong Wang**, Li Li, Zhizhen Zhang and Dingsheng Li, University of Nevada, Reno*

Chemicals present in the environmental and occupational settings can exert adverse health effects on humans after gastrointestinal absorption into the systemic circulation. Parallel Artificial Membrane Permeability Assay (PAMPA) has gained advocacy for its application to measure the effective permeability towards pharmaceuticals. However, since chemicals of environmental or occupational concerns differ from pharmaceuticals in hydrophobicity and volatility, a thorough, mechanistic understanding of chemical mass transfer in PAMPA is warranted if we seek to expand the applicability of PAMPA. Here, we introduce an in-silico mass balance model, which describes chemical mass transfer in PAMPA based on inputs of fundamental physicochemical properties, e.g., molecular weight, partition coefficient, and dissociation constant. The model's performance is evaluated by an agreement between predicted and measured permeabilities of 1383 chemicals, which indicates that 95% of the estimated permeabilities are either fall in the same order of magnitude or more conservative than the measurements. The model predicts an inverted U-shaped dependence of permeability on the octanol-water partition coefficient (log $K_{\mathrm{OW}}$ for neutral compounds and log $D_{\mathrm{OW}}$ for ionizable compounds), with the maximum permeability occurring in log $K_{\mathrm{OW}}$ or log $D_{\mathrm{OW}}$ ranging between 0 and 2. The model estimates a high membrane retention rate for hydrophobic chemicals, as well as the loss of volatilization to the headspace of the PAMPA apparatus for highly volatile chemicals. Notably, the measured permeabilities of hydrophobic chemicals are remarkably sensitive to specific experimental conditions, e.g., frequency of stirring, and incubation time, making measurements under different conditions less comparable. More important, for highly hydrophobic chemicals (log $K_{\mathrm{OW}}$ or log $D_{\mathrm{OW}}$ greater than 3.8), steady-states mass transfer, which is the fundamental assumption of PAMPA can never be achieved. Therefore, the current design of PAMPA needs to be modified before its application to chemicals of environmental or occupational concerns. Our work provides an in-silico mechanistic approach in support of efficiently and defensibly predicting the permeability of chemicals and complements the current laboratory approach.

## 5.11.V In silico NAMs: Recent Developments and Regulatory Applications

### 5.11.V-02 Machine Learning Models for Predicting Human Liver Microsomal Metabolism of Organophosphate Pesticides

***Thomas Lane**[1], Fabio Urbina[1], Garima Agarwal[2], Craig McElroy[2] and Sean Ekins[1], (1) Collaborations Pharmaceuticals, Inc., (2) The Ohio State University*

Accidental exposure and intentional poisoning by organophosphate pesticides (OPPs) kill hundreds of thousands of people every year. Over the past 20 years, large quantities of in vitro and in vivo data have accumulated on drug metabolism in humans. Machine learning methods have been applied to many of these datasets for toxicological research to enable prospective prediction and increase efficiency of screening. The availability of drug metabolism data enables the building of predictive computational models using molecular structure. Previously, we had curated data for human liver microsome (HLM) metabolism from public sources, followed by building of machine learning models with our Assay Central software. These models were used to predict the clearance for 36 OPPs and these were compared to *in vitro* values, but these showed no predictive ability. Since this more traditional modeling approach showed poor predictive ability for OPP metabolism, likely due to the lack of representative OPP in the training sets, we have now expanded our methodology. Firstly, as many of the OPP clearance curves did not follow the expected decay curve of a first order reaction, we extended our modeling approach. Most QSAR revolves around predicting single point values of a molecule-target interaction. Single-point predictions do not take advantage of the full dose-response curve often generated for these drug-target interactions by collapsing these data into a single-value with potentially critical information loss. Recently, we have shown the ability to predict UV-vis spectra curves, which is continuous data, from molecular structure using attention-based recurrent neural networks. We've applied this same architecture to predict full dose-response curves based on molecule structure allowing us to predict not only the clearance of these molecules, but also the maximal response as well as the response at different concentrations. Secondly, we have compared the experimental clearance rates for 18 OPPs in HLM with those predicted by relative activity factor (RAF) mathematical models. These RAF models used individual CYP metabolism experimental data to predict microsomal clearance. This approach performed reasonably well, suggesting these individual CYP models could be used in conjunction with the HLM clearance models. Based on this we also utilized a LSTM approach to incorporate both individual CYP and HLM metabolism data to predict HLM metabolism.

### 5.11.V-04 Performance Comparison of Acetylcholinesterase Inhibition Machine Learning Models for Multiple Species

***Patricia Vignaux***, *Fabio Urbina, Thomas Lane, Ana C. Puhl, Sean Ekins and Jacob W. Gerlach, Collaborations Pharmaceuticals, Inc.*

Acetylcholinesterase (AChE) is an important target for commercial pesticides and parasiticides. Overdose or exposure to these AChE inhibitors can result in accidental or intentional poisoning. Due to this, AChE inhibitors can be potential pollutants and AChE activity is an oft-used biomarker in environmental toxicology. Recently, there has been an interest in identifying potential new environmental pollutants that inhibit AChE but which may fall outside of the traditional category of carbamate and organophosphate pesticides. We have developed machine learning models for AChE inhibition in seven different species, using publicly available data from ChEMBL and BindingDB. We built consensus classification models comprised of eight different algorithms for each species using ECFP6 fingerprints, with an activity cutoff of 1 μM. Consensus models performed well with five-fold internal cross validation, and in particular the human (4061 compounds) and eel (5424 compounds) consensus models predicted activity in human and eel external test sets with 78% and 83% accuracy, respectively. However, the reciprocal cross (74% and 83% percent accuracy) showed a lack of species specificity in our models. We saw better species specificity in our regression models, where our human Support Vector Regression model of human AChE inhibition (3652 compounds) predicted the $IC_{50}$'s of the human test set (Pearson correlation $r = 0.77$ and $R^2 = 0.59$), compared to the eel regression model (4930 compounds) on the same test set (Pearson correlation $r = 0.62$, $R^2 = 0.38$). However, the predictive power of these models decreases as the distance from the chemical space found in ChEMBL and BindingDB increases. In an effort to expand our models, we have incorporated data from a recent screen of Tox21 compounds into our human regression and classification models (3825, 10391 compounds). We are currently also combining all our datasets to create a pan-vertebrate AChE inhibition model. Our goal is to incorporate as much chemical space as we can, in order to be able to identify potential AChE inhibitors among molecules found on the NORMAN Suspect List Exchange. The advantage of our machine learning approach is the ability to rapidly score metabolites or other compounds that can then be selected for future *in vitro* testing. Our ultimate goal is to create a suite of AChE models for predicting direct inhibition of AChE that can be delivered as an easy-to-use application as part of a commercial product.

## 5.12.P Leveraging and Adapting Environmental Methods and Monitoring Tools for COVID-19 Public Health Research and Surveillance

### 5.12.P-We170 Spatial Associations of Long-term Exposure to Diesel Particulate Matter with Seasonal and Annual Mortality Due to COVID-19 in the Contiguous United States

***Martine Mathieu*** *and Jennifer Richmond-Bryant, North Carolina State University*

The Center for Disease Control and Prevention stated that people with certain underlying respiratory and cardiovascular conditions (chronic obstructive pulmonary disease, lung cancer, asthma) might be at an increased risk for severe illness from coronavirus disease 2019 (COVID-19). Diesel Particulate Matter (DPM) exposure may affect the pulmonary and cardiovascular systems. The objective of the study is to assess if long-term DPM exposure was spatially associated with COVID-19 mortality across three consecutive waves (January-May, June-September, October-December) of the disease and throughout 2020. We tested a series of association models, starting with an ordinary least square model. After observing spatial dependence in the residuals, two models designed to explore spatial dependence across the domain, a spatial lag model and a spatial error model, and a geographically weighted regression (GWR) model designed to explore local associations, were run to analyze spatial patterns and seasonal relationships between COVID-19 mortality and long-term exposure to DPM. Positive associations were observed for the January-May and June-September waves with all three global models. The local GWR model found that associations between COVID-19 deaths and DPM concentrations may increase up to 57, 36, 43, and 58 deaths per 100,000 people in some US counties for every 1 $\mu g/m^3$ increase in DPM concentration for the January-May, June-September, October-December, and yearlong models. Relative significant positive association are observed in New York, New Jersey, eastern Pennsylvania, and western Connecticut for the wave from January to May, and in southern Florida and southern Texas for June to September. The period from October to December exhibit a negative association in most parts of the US, which seems to have influenced the year-long relationship due to the large number of deaths during that wave of the disease. Our models provided a picture in which long-term DPM exposure may have influenced COVID-19 mortality during the early stages of the disease, as observed specifically for the periods of January-May and June-September 2020. Waning influence of DPM during October to December suggested that person-to-person disease transmission regardless of past exposures may have become more influential in the spread of COVID-19 and in mortality rates once the disease became widespread throughout the U.S.

**5.12.P-We171 Identification of Potential SARS-CoV-2 Intermediate Host Species Using SeqAPASS**

***Sally A. Mayasich***[1] *and Carlie LaLone*[2]*, (1) University of Wisconsin, Madison, (2) U.S. Environmental Protection Agency*

COVID-19 is a zoonotic disease caused by infection of the coronavirus SARS-CoV-2. SARS-CoV-2 has been demonstrated to pass from animals to humans and from humans to animals; infections have been detected in animals in North America including minks, ferrets, white-tailed deer, big cats, and domestic animals. SARS-CoV-2 enters the cells of host animals and replicates to transmissible levels by evading host innate immune responses through a variety of interactions with host proteins. The viral spike protein binds to the angiotensin-converting enzyme 2 (ACE2) protein as a receptor on the host cell surface to enter the cell. The virus uses the host cell machinery to translate its RNA genome into proteins that block host proteins in the interferon-I (IFN-I) antiviral pathway, a crucial process allowing the virus to replicate. Understanding conservation of these host antiviral proteins across species could aid in determining which species are likely to serve as reservoirs or intermediate hosts. The web-based US EPA Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) tool facilitates the comparison of proteins to evaluate conservation across hundreds of species rapidly. In this novel application, critical domains and amino acids involved in protein-protein interactions between viral proteins and human host antiviral proteins were identified. Conservation of antiviral protein sequences across species compared to the human proteins was evaluated to assess their vulnerability to attack by SARS-CoV-2 viral proteins. From a literature review, IFN-I antiviral pathway protein targets such as mitochondrial antiviral signaling protein (MAVS), IFN regulatory factor 3 (IRF3), and IFN-stimulated gene 15 (ISG-15) were selected for evaluation. The SeqAPASS Level 1 primary amino acid sequence comparison indicated a lack of conservation of these proteins in non-mammals. Further, functional domains and critical amino acids in the human IFN-protein-to-viral-protein interaction sites were queried and results were evaluated from species that had previously been studied empirically for COVID-19 infectability or bioinformatically for ACE2 binding potential. A list of mammals exhibiting structural conservation for these proteins could then be mapped to geographic regions across the United States to inform zoonotic disease surveillance efforts. *This abstract neither constitutes nor necessarily reflects USEPA policy.*

**5.12.P-We172 Long-term SARS-CoV-2 Wastewater Surveillance for Two Locations in Texas, USA Compared to COVID-19 Epidemiology Data**

***Megan O'Brien***[1]*, Laura M. Langan*[1]*, Lea Marie Lovin*[1]*, Kendall Rose Scarlett*[1]*, Abigail Henke*[1]*, Haley Davis*[1]*, Fallon Bain*[1]*, Christine Snow*[1]*, Aubree Miller*[1]*, Sean Norman*[2]*, Heidi K. Bojes*[3]*, Sarah E. Seidel*[3]*, Natalie*

*Archer[3], Eric Lawrence[3] and Bryan W. Brooks[1], (1) Baylor University, (2) Arnold School of Public Health, (3) Texas Department of State Health Services*
Due to its rapid emergence and subsequent global spread in late 2019, severe acute respiratory coronavirus 2 (SARS-CoV-2) rapidly became a health threat, affecting people worldwide. Due to limited personnel and supply chain deficiencies, traditional clinical testing methods provided an inconsistent approach for evaluating community spread of COVID-19. Because SARS-CoV-2 RNA can be detected in feces of infected individuals prior to exhibiting symptoms, wastewater-based epidemiology (WBE) became a tool that can track trends in SARS-CoV-2 RNA loads for the desired population, where surges in viral loads can indicate potential surges in clinical cases in the following days. We characterized two different areas in Texas over a period of 14 months quantifying SARS-CoV-2 wastewater RNA concentrations, along with COVID-19 related epidemiological data specified for each population. Throughout the sampling period, weekly 24 hr. composite raw wastewater influent was collected in influent from two comparable regional wastewater treatment facilities in Denton and Waco, Texas, and examined wildtype (N1) viral loads using RT-qPCR. Epidemiological data was confirmed and provided by the Texas Department of State Health Services. Relationships between wastewater viral loads and epidemiological data for COVID-19 cases, intensive care unit (ICU) admissions, and mortalities in both populations were subsequently examined. Increases in wastewater viral loads preceded increases in positive clinical tests, ICU admissions, and mortalities, though the strength of the association varied between locations. Relationships between new clinical cases and wastewater SARS-CoV-2 RNA concentrations were calculated using Pearson correlation and linear regression analysis with 0-, 7-, 14-, and 21-day rolling averages. The 21-day calculated rolling average demonstrated the highest correlation with wastewater RNA for both Denton and Waco, Texas ($R = 0.55$ and 0.79, respectively), indicating smoothing techniques for epidemiological data may be useful for reducing daily variability of clinical testing data available to WBE researchers.

**5.12.P-We173 Assessment of a mass balance equation for estimating community-level prevalence of COVID-19 using wastewater-based epidemiology in a mid-sized city**

***Andrew L. Rainey**[1], Julia C. Loeb[1], Sarah Robinson[1], Song Liang[1], Paul Davis[2], John A. Lednicky[1], Eric Coker[1], Tara Sabo-Attwood[1], Joseph H. Bisesi Jr[1] and Anthony T Maurelli[1], (1) University of Florida, (2) Gainesville Regional Utilities*
Wastewater-based epidemiology (WBE) has emerged as a valuable epidemiologic tool to detect the presence of pathogens and track disease trends within a community. WBE overcomes some limitations of traditional clinical disease surveillance as it uses pooled samples from the entire community, irrespective of health-seeking behaviors and symptomatic status of infected individuals. WBE has the potential to estimate the number of infections within a community by using a mass balance equation, however, it has yet to be assessed for accuracy. We hypothesized that the mass balance equation-based approach using measured SARS-CoV-2 wastewater concentrations can generate accurate prevalence estimates of COVID-19 within a community. This study encompassed wastewater sampling over a 53-week period during the COVID-19 pandemic in Gainesville, Florida, to assess the ability of the mass balance equation to generate accurate COVID-19 prevalence estimates. The SARS-CoV-2 wastewater concentration showed a significant linear association (Parameter estimate = 39.43, P-value<0.0001) with clinically reported COVID-19 cases. Overall, the mass balance equation produced accurate COVID-19 prevalence estimates with less than 1% (P-value = 0.27) difference from our clinical reference group. Therefore, the mass balance equation applied to WBE is an effective tool for generating accurate community-level prevalence estimates of COVID-19 to improve community surveillance.

**5.12.P-We174 Analysis Of The Fate And Transport Of SARS-CoV-2 In Wastewater And Surface Waters In The United States Using iSTREEM®**

***Raghu Vamshi**[1], Brenna Kent[1], Scott Dyer[1], Ryan E. Heisler[2] and Kathleen Stanton[2], (1) Waterborne Environmental, Inc., (2)American Cleaning Institute*
The ongoing pandemic of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is taking a huge toll on humankind.  Infected people excrete the virus through

their feces which is conveyed to wastewater treatment plants (WWTP) where its genetic material, RNA, can be detected. SARS-CoV-2 may remain active while being transported in water through the WWTP and into receiving waters. Therefore, it is critical to determine the distance the virus may travel and whether surface water, including drinking water, is at risk. Modeling the fate of viruses in WWTPs and surface water on a national level could be an additional evaluation of monitoring efforts. iSTREEM®, a tool used for modeling the fate and transport of down-the-drain materials, was used to estimate viral concentrations in effluent at WWTPs and surface water for the continental U.S. Inputs for modeling included the viral load, removal in WWTPs, and in-river decay which were based on monitoring data, current literature, and expert opinions. This analysis indicated that WWTPs are highly efficient in removing SARS-CoV-2. Residual RNA fragments were either removed or diluted in the surface waters and were not measured above current detection limits. Treatment of drinking water will result in even greater loss of viral fragments, if present, indicating that SARS-CoV-2 most likely does not pose a health risk in the U.S. via drinking water. This is the first study to provide quantitative data at a national scale to support these claims.

## 5.13.P Microplastics in the Environment and Risk Assessment: A One-Health Perspective

### 5.13.P-Th097 Sampling and Analysis of Airborne Microplastic Particles from a Materials Recovery Facility

***Diana L. Ortiz-Montalvo***, *Abigail P. Lindstrom, Joseph M. Conny, Thomas P. Forbes, John M. Pettibone and Eric S. Windsor, National Institute of Standards and Technology (NIST)*

Microplastic and nanoplastic (MNP) particles have gained attention for their occurrence in our environment and the many unknowns about their negative health impacts. Only limited data currently exists about potential exposures and effects of airborne MNPs. Moreover, there is a lack of validated methods for sampling and identifying airborne MNPs. This work aims to develop protocols for identifying and measuring airborne MNP particles to better understand health risks from exposure via inhalation. The project will allow us to determine how well MNPs can be distinguished from common dust-like particles and among different polymers (e.g., polystyrene, polyethylene, polypropylene, etc.). We couple a novel air sampling methodology with optical, electron, and Raman microscopy for microplastic detection and chemical identification.

A commercially available sampler was installed at a materials recovery facility (MRF) to collect airborne particles on membrane filters. The sampler was installed proximate to where recycled materials (i.e., glass, metals, and plastics) were unloaded on conveyor belts, and workers removed unrecyclable objects. Particles released from materials introduced into the MRF were sampled, including MNPs from plastic products. Samples consisted of particles collected on polycarbonate filters pre-coated with aluminum and pre-marked with an innovative fiducial pattern. The aluminum layer provided a low background signal for micro-Raman spectroscopy. The fiducial pattern aided in sectioning the filter and locating the particles of interest between the different microscopy instruments. Operating conditions (e.g., flow rate and sampling times) were varied to gauge the filter loading for optimizing analysis work. Light microscopy is used to identify candidate MNPs by color and inspect the filter loading. Scanning electron microscopy and X-ray (SEM/EDS) analysis are used to distinguish carbonaceous particles from non-carbonaceous materials like glass and metal. Micro-Raman spectroscopy is used to identify types of polymers based on reference spectra from cryomilled reagent plastics and library spectra. Additionally, we are exploring other state-of-the-art microanalytical techniques for the samples, such as time-of-flight secondary ion mass spectrometry (TOF-SIMS) and optical photothermal infrared spectroscopy (O-PTIR). The measurement tools and methods necessary to accurately identify MNPs in applied systems will likely have to include new protocols.

### 5.13.P-Th094 Microplastic Taxonomy: Harmonizing Microplastic Classification

***Hannah Hapich***, *Win Cowger and Andrew Gray, University of California, Riverside*

Microplastics pose toxic threats to humans, wildlife, aquatic organisms, and ecosystems. With growing concerns over the types of effects microplastics can have on living organisms and air, water, and soil quality, the number of studies quantifying and classifying environmental microplastic pollution has increased exponentially over the past five years. The large and diverse datasets resulting from these studies have the potential to inform and advance the science and management of microplastic pollution on a massive scale. However, our study found that much of the microplastic research data collected to date is not is not comparable in its current state due to differences in classification systems used for color, morphology, sizes, and material types. We surveyed the literature and developed a new suite of relational tables, similar to the Trash Taxonomy Tool, however focusing specifically on the harmonization of microplastics datasets. This Microplastics Taxonomy Tool provides a framework to relate microplastic classification terms by their alias (synonymous) and hierarchical (nested) terms. We summarize the prevalence of the terms currently used in the literature and propose a path for the field to improve microplastic categorization. The microplastic taxonomy will allow users ranging from researchers to policymakers to easily compare and harmonize existing microplastic studies.

**5.13.P-Th098 Characterization and Quantification of Microplastic Concentrations in the Benthic and Pelagic Habitats of the San Pedro Shelf.**

***Samiksha Singh***[1]*, Hannah Hapich*[1]*, Win Cowger*[1]*, Karen McLaughlin*[2] *and Andrew Gray*[1]*, (1) University of California, Riverside, (2) Southern California Coastal Water Research Project (SCCWRP)*

Microplastic monitoring in the coastal environment has primarily focused on the sea surface. However, sampling throughout the water column is important because many manufactured plastics are negatively buoyant and positively buoyant particles can be found at depth because of vertical mixing. Temporal variability introduced by seasonal inputs (i.e. fluvial fluxes) as well as the spatial variability of nearshore and offshore particle transport dynamics are important controls on the concentration of microplastics in coastal environments that need to be explored. To address these knowledge gaps, a microplastic monitoring campaign was carried out in the coastal waters of the San Pedro Shelf in the Southern California Bight. Sampling occurred in both winter and summer to investigate the impacts of differences in circulation, stratification, and fluvial inputs. Sampling locations included transects inshore and offshore of the Los Angeles River and the San Gabriel River mouths and three locations along the Huntington Beach coastal shelf. Each site's transect included monitoring at the surface (manta trawl), mid-water column (Bongo net) and the bottom (Epibenthic sled). Microplastics extracted from samples were characterized in terms of color, morphology, and particle dimensions. A subset was further analyzed for polymer type using spectroscopic techniques. Preliminary results indicate that greater microplastic concentrations were found in the nearshore environments during the winter season when there is a larger input from land-based sources via episodic rainfall events. The ambient monitoring of inshore and offshore benthic and pelagic environments will provide important data to inform transport modelling, source, fate, and transport evaluation, and eventual assessment of risks to marine health.

**5.13.P-Th099 Specific profiles of organic additives in bioplastic products collected from Indonesia, Japan, Myanmar and Thailand and implication for their environmental exposure**

*Nur Latifah*[1]*, Thant Zin Tun*[1]*, Suchana Chavanich*[2]*, Voranop Viyakarn*[2]*, Atsuhiko Isobe*[3] *and* ***Haruhiko Nakata***[1]*, (1) Kumamoto University, Japan, (2) Chulalongkorn University, Thailand, (3) Kyushu University, Japan*

To understand the occurrence and profiles of polymers and organic additives in biomass- and biodegradable plastic products, thirty-two plastic bags collected from Indonesia, Japan, Myanmar, and Thailand during 2021 and 2022 were analyzed. The results of FTIR suggested that polyethylene (PE) was used in all biomass, oxo-biodegradable, and LIMEX plastic bags, whereas 23% of biodegradable-labelled plastic bags were PE. Starch was the major polymer used in biodegradable plastics, especially in Indonesia, which accounts for 62%, followed by copolyester of 1,4-butanediol for 15%. Plastic additives profiles in samples showed that biomass plastics were almost similar to that of LIMEX plastics, consisting of a minimum of 90% antioxidants. In contract, oxo-biodegradable plastics and biodegradable plastics having smooth surface were proven to have 26-56% lubricants of overall composition.

As for organic additive analysis, plasticizer of bis(2-ethylhexyl) phthalate (DEHP), antioxidant of irgafos 168 and irganox 1076 were prevalent in all PE plastic bags (biomass, oxo-biodegradable, and LIMEX) from Japan, Indonesia, and Thailand. Butylated hydroxytoluene (BHT) and Irganox 1076 were mainly detected in Myanmar oxo-biodegradable plastic bags. On the other hand, starch plastic bags mainly contained lubricants, such as erucamide and oleamide and plasticizer (TXIB). Starch plastic bags, made from cassava, have been commercially promoted to be easily dissolved in hot water and be readily to drink which make it safe to the environment. However, dissolving the plastic bag released higher amount of additives including dodecanoic acid, *n*-hexadecanoic acid, 1-hexadecanol, 10E,12Z-octadeca-10,12-dienoic acid, oleamide, and erucamide. Moreover, one biodegradable sample from Japan was found to contain vast amount of irganox 1076, erucamide, and tributyl acetylcitrate which made it the most containing plastic additives among all samples.

To understand specific profiles of organic profiles in bioplastics, PCA analysis was performed based on compositions of individual peak area of GC-MS chromatograms. Interestingly, there are 3 groups: biomass plastic bags (blue), oxo-biodegradable plastic bags (pink), and biodegradable plastic bags (grey) were identified, implying that same types of plastic bags have similar organic additives profiles.

**5.13.P-Th100 Microplastics Quantification in Edible Bivalves From the Lagoon of Venice Using Nile Red Staining**

***Davide Asnicar**[1,2], Alberto Santi[1], Fabiana Corami[3], Albino Gallina[4], Carmen Losasso[4] and Maria Gabriella Marin[1], (1) University of Padova, Italy, (2) Huntsman Marine Science Center, Canada, (3) Institute of Polar Science - National Research Council (ISP-CNR), Venezia-Mestre, Italy, (4) Istituto Zooprofilattico Sperimentale delle Venezie, Italy*

Microplastics (MPs) are increasingly present in aquatic environments worldwide, reaching coastal areas and oceans as the ultimate sink. Therefore, marine organisms, and in particular filter-feeders such as bivalves, are continuously exposed to MPs, which can then enter the food web. In addition, MP accumulation in edible bivalves could represent a health risk for humans. In the present project, the distribution of MPs in the Lagoon of Venice was assessed using two marketable bivalves, the mussel *Mytilus galloprovincialis* and the clam *Ruditapes philippinarum*, as sentinel organisms. In the environment, the two species are differently exposed to MPs depending on the density and aging of the particles. While mussels filter in the water column, clams are infaunal animals filtering resuspended sediments where MPs accumulate.

This sampling campaign provided a comprehensive picture of the MP contamination level in the Lagoon. To assess the presence of MPs extensively, mussels and clams were sampled in 19 and 8 sites, respectively, covering the entire area of interest. To extract MPs, animals were digested with $H_2O_2$ (for mussels) and KOH (for clams). The slurry was filtered on 5 µm PVDF filters and stained with Nile Red solution to reveal MPs under UV light. To validate the method, the results from the Nile Red staining were compared with the µ-FTIR technique performed on subsamples of animals from the same sites. Analyses showed that MPs were present in all the bivalve samples. The highest number of MPs/individuals were found in the areas characterized by high anthropogenic influence, compared to more remote areas of the Lagoon.

Based on the wide size range available for mussels, different dimensional classes were considered for this bivalve. Higher MPs content was found in larger sized animals, suggesting bioaccumulation. However, the ratio of MPs number/wet weight was higher in smaller animals. This result highlights the need to properly consider the animal size in MPs quantification studies.

**5.13.P-Th102 Possible exposure risks to metal additives in 3D printing thermoplastics**

***Anna M. Wade**[1], Todd Luxton[2] and Derek Peloquin[3], (1) Oak Ridge Institute for Science & Education at US EPA, Cincinnati, (2) U.S. Environmental Protection Agency, (3) U.S. Food and Drug Administration*

Manufacturing advancements in three-dimensional (3D) printing of plastic polymers have outpaced our understanding of exposure risks for domestic use. One understudied aspect is the release of metal additives incorporated into plastic polymers before and after printing. Metal additives are not chemically bound to the plastic polymer and given that 3D printed objects have longer lifespans than single-use plastics, the potential for human exposure to these additives needs study. Here, we analyzed pre-ingestion human exposure risks from PLA and PLA/PHA thermoplastic filaments infused with copper, bronze, and stainless-steel fine metal powders. Raw and printed filaments were subjected to 2-hour release scenarios that simulated contact with water, synthetic sweat and synthetic saliva. Concentration, particle size and speciation of metal additives in the thermoplastics and leachates was determined using total metals analysis, sequential filtration, electron microscopy, and X-Ray Absorption Fine Structure Spectroscopy. Estimates on the impact of plastic degradation on metal additive release, for raw and extruded filaments, were accomplished by exposing thermoplastics to UV exposure equivalent to yearly-average insolation in Cincinnati, OH. The quantity of copper (Cu), tin (Sn), and chromium (Cr) released during 2-hour leaching periods were highest in synthetic sweat followed by synthetic saliva and water. Additionally, migratable concentrations of Cu, Sn, and Cr were higher in raw filament than printed filament, and higher in PLA/PHA plastic than PLA plastic. The quantity of Cu and Cr leached from the thermoplastics were higher than USEPA permissible limits of 1.3 mg/L for Cu and 0.1 mg/L for Cr in drinking water, with maximum concentrations topping 327 ppm and 7.0 ppm for Cu and Cr respectively. Photo degradation by UV weathering increased migratable concentrations of Cu, Sn, and Cr by one to two orders of magnitude. Leached metals were in the particulate phase with size fractions below 150 µm, indicating high potential for dermal adherence and subsequent ingestion. Our findings suggest dermal contact and subsequent ingestion with raw filament is a possible exposure pathway when working with metal additive 3D printing filaments. Appropriate filament storage should be used to minimize photo degradation of filament over time and proper filament handling to reduce incidental exposure during the printing process.

**5.13.P-Th104 Floating Microplastics in the St. Lawrence River in Canada**

***Juan Manuel Gutierrez-Villagomez*[1]*, Tuan Anh To*[1]*, Ève Larocque*[1]*, Adam-Emmanuel Tremblay*[2] *and Valerie S. Langlois*[1]*, (1) Institut National de la Recherche Scientifique (INRS), Canada, (2) Université Laval, Canada*

Microplastics (MPs) are synthetic or semisynthetic polymers ubiquitously found in freshwater ecosystems and potentially deleterious to living organisms. In this project, we quantified and characterized the floating MP fraction at 11 sites through the St. Lawrence River (SLR). The SLR forms the primary drainage outflow of the Great Lakes Basin to the North Atlantic Ocean and crosses the province of Quebec in Canada. Sampling occurred from May to July 2021 using two sampling nets with different sizes, structural construction, and mesh size: Manta (40 × 20 cm rectangle metal frame with a 300-µm mesh net) and Poly-Mer (33 × 15 cm diameter rectangle wooden frame with a 100-µm mesh net). The nets were towed in parallel from a boat for 20 min in the top 50 cm from the surface at each of the sampling sites (n = 2-4). Samples were prepared and analyzed for fibres, plastic fragments, and spheres using an optical microscope. Each particle was analyzed using Fourier Transform Infrared Spectroscopy (FTIR), then compared to the library standards for compound annotation. Preliminary data confirmed the presence of MPs at each of the sampling sites along the SLR with MP concentrations ranging from 125 to 861 particles of plastics per million litres (PPML) according to the studied site. The most abundant categories of MPs found were the fibres, followed by the fragments, and lastly, the spheres. The FTIR analysis showed that polyester, polyethylene, polypropylene, nylon, and polystyrene were the five main types of MP material found in the SLR for both nets. This work provides valuable information about the distribution of MPs in aquatic ecosystem in North America for water resource protection and management.

**5.13.P-Th105 Toxicity of Polyvinyl Chloride Microplastics and Plastic Additive Dibutyl Phthalate on the Green-Lipped Mussel, Perna canaliculus, After Individual and Combined Exposure**

***Camille Gabriella Baettig*[1,2]*, Amy Ockenden*[1]*, Kirsty F. Smith*[2]*, Gavin Lear*[1]*, Grant Northcott*[3] *and Louis Tremblay*[2]*, (1) University of Auckland, New Zealand, (2) Cawthron Institute, New Zealand, (3) Northcott Research Consultants Ltd, New Zealand*

Microplastics (MPs) are now ubiquitous in marine environments occurring from surface waters to sediments and are an increasing concern for filter feeding organisms. Plastic polymers have an assortment of compounds added to alter their properties during manufacture, e.g., plasticizers such as dibutyl phthalate (DBP). Most additives are physically bound to plastic rather than chemically, leading to leaching from plastic into the aquatic environment or into organisms after ingestion. Because of this leaching effect, we propose that MP can potentially harm the organism via three mechanisms: 1) through the physical MP interaction with organisms, 2) the toxic effect of additives leaching from the MP, or 3) a combined effect of MP and additive. DBP was selected as is one of the most toxic plasticizers and has been found in numerous aquatic environments. The aim of this study is to elucidate the effects of polyvinyl chloride (PVC) MPs alone, plastic additive DBP alone, and of PVC MPs with DBP incorporated. Pristine PVC plastic sheets and PVC sheets made with DBP at 30% sample weight were manufactured and then cryoground to MPs with a size range of 63 – 90 µm. Early adult green-lipped mussels, *Perna canaliculus*, were exposed to pristine PVC MPs (1000 $\mu g\ L^{-1}$), DBP (300 $\mu g\ L^{-1}$), and PVC MP with DBP incorporated (1000 $\mu g\ L^{-1}$) during manufacturing for seven days. To understand the potential mechanisms and modes of action behind both polymer and contaminant stressors whole transcriptome analysis will be performed. Preliminary experiments exposing mussels to MPs and DBP alone were used to determine concentrations for transcriptomic samples. After seven-day exposure to multiple concentrations of MPs, significant modulation of oxidative stress genes, CAT and SOD, as well as generic stress marker HSP70 was seen in the gill tissue at 1000 $\mu g\ L^{-1}$. Corresponding DBP concentrations (30% v/w) were used, and significant modulation was seen at concentrations ranging from 30 – 450 $\mu g\ L^{-1}$ in CAT and SOD in the gills. No significant results were seen in the digestive gland after either exposure. By implementing the transcriptomic approach, we will gain insight into the effects of PVC MPs and DBP across the whole genome, allowing for quantitative assessment of these changes and discovery of novel pathways affected by MPs and DBP in *P. canaliculus*. These results will provide invaluable knowledge that can be extrapolated to other MPs and phthalate plasticizers for future studies.

**5.13.P-Th107 The most effective, efficient and least destructive method(s) for extracting microplastics from complex water samples**

***Razegheh Akhbarizadeh*[1]*, Jessy Xu*[1]*, Katie Wang*[1]*, Miriam L. Diamond*[1] *and Paul Helm*[2]*, (1) University of Toronto, Canada, (2) Ontario Ministry of the Environment, Conservation and Parks, Canada*

Microplastic and other anthropogenic particles (e.g., waxes, tire wear, paints) are extracted from complex water samples with a variety of purification methods. However, some protocols are not necessarily efficient in eliminating organic matter, especially in samples with high amounts of organic matter and other interfering matrices. In addition, some polymers (e.g., polyamide fibers and films) are susceptible to degradation during the digestion process. Therefore, we have undertaken a systematic investigation of the effectiveness of extraction procedures to more completely remove the unwanted matrix while balancing the retention of microplastic particles, using complex urban stormwater runoff samples as a test matrix. Sequential combinations of oxidative-alkaline protocols (e.g., potassium hydroxide, hydrogen peroxide, Fenton's digestion) were applied to the complex organic matrix from urban runoff samples and to particles comprised of a range of polymers (olefins, polystyrene, polyamide, polyesters, tire rubber) and a variety of sizes and shapes (with and without interfering matrices). Initial tests showed that combinations of the oxidative-alkaline digestions were more effective for eliminating organic matter than Fenton's digestion alone (88-95% vs. 77% mass reduction of solid materials) while reducing operator time per sample. However, our results also showed that protocols that are more effective at reducing organic matter may degrade some targeted polymers. For example, polyamide fibers that underwent hydrogen peroxide digestion (15 and 30% solutions) showed complete dissolution, but degradation was minimal at a very low concentration (3%) after 24h exposure at 55 degrees Celsius. We will present protocols that balance efficiency (time and effort) and effectiveness (maximize removal of organic matrix) while also minimizing degradation of targeted polymers suitable for 63-1000 µm size range.

**5.13.P-Th108 Effects of three micro and nanoplastics under weathering conditions on messenger and long non-coding RNA expression in the Inland Silverside (Menidia beryllina)**

***Sara J. Hutton***, *Samreen Siddiqui, John Dickens, Emily Pedersen, Bryan J. Harper, Stacey L Harper and Susanne M. Brander, Oregon State University*

Micro and nanoplastics (MNPs) are the result of widespread plastic pollution which has become an environmental pollution issue of great concern. As plastics breakdown into smaller fragments the addition of weathering processes can further aid in degradation as well as alter the toxicity of the particles. However, MNP toxicity is still poorly understood and the potential mechanisms of toxicity for this class of compounds has yet to be fully explained. Additionally, how toxicity may be altered following weathering processes is even less well studied. Therefore, to better understand the potential differences in mechanisms of toxicity across MNP types, shapes, sizes, and weathering processes we have exposed the Inland Silverside (*Menidia beryllina)* to three different MNP types: tire particles (TP) and poly-lactic acid (PLA) at both micro and nano size fractions and polyester microfibers (MFs) in the micro size fraction to both weathered and un-weathered forms. MNPs were weathered for 72 hours with exposure to UV A, B, and C while being constantly shook in saltwater. Organisms were exposed to either weathered or un-weathered particles for 21 days. Following the exposure, RNA was extracted and is currently in the process of being sequenced for both messenger and long non-coding RNA (mRNA and lncRNA respectively). Following sequencing, bioinformatic analysis will be used to determine differentially expressed genes. mRNA will inform on the gene expression of the organisms following chronic exposure which will allow for an assessment of differentially expressed gene pathways. lncRNA play essential biological roles and are additionally important in development of diseases, the analysis of which will also provide insights into the potential epigenetic effects of MNPs. Here, we present our experimental design as well as early preliminary results which will be available at the time of presentation. This data will provide a necessary comparative assessment of multiple MNP types to better understand the relative toxicity of these contaminants. Additionally, this will be the first assessment of lncRNA's in the Inland Silverside, a commonly used model species in both research and regulatory testing.

**5.13.P-Th109 Characterization of Airborne Micro-plastics in Coastal Areas**

***Joshua Ugor****, Mike Fowler and Fay Couceiro, University of Portsmouth, United Kingdom*

Micro-plastics (MPs) are defined as synthetic solid particles or polymeric matrices, with regular or irregular shape and with size ranging from 1µm to 5mm. Indiscriminate dumping of plastic waste in the environment and water bodies which is acted upon by ultraviolet rays, hydrological factors and other environmental conditions leads to the weathering of these plastics waste resulting in movement across compartments of the environment gives rise to significant levels of micro-plastic pollution. Due to the limited information/research on characterization of airborne micro-plastics in coastal/marine environment for micro-plastics less than 20 µm, it becomes important that the characterization of atmospheric micro-plastics in these areas be investigated. As a better understanding may eventually assist in mitigation of plastic contamination of the entire environment and alleviate pressure on human and ecosystem health. The scope of this study includes; The characterization of micro-plastics in coastal/marine environments of United Kingdom, Evaluation of the dynamics of micro-plastic in the coastal/marine environment as it relates to sea spray, significance of the pathway for plastic cycle toxic transport in coastal/marine environment and its effects on living organisms (aquatic and terrestrial life). This project aims to assess the extent of micro-plastic pollution in coastal/marine environment in UK examining the micro-plastics sources, size distribution, transport and fate. The project will be achieved through; Measurement & Characterization of micro-plastics in different coastal/marine environment,Quantification, Understanding the seasonal variation in micro-plastic distribution. Samples will be gotten from 5-8 randomly selected coastal/marine areas within UK using a deployable particulate sampler. Samples will be properly prepared for further analysis using the Raman spectrometer and a Scanning electron microscope to further ascertain the properties & characteristics. Literature search to compare results, the team will review the result which will aid in the production of a poster and report. The effect of the impact of both South Atlantic and North Atlantic ocean to micro-plastic distribution will be ascertained. The study will also enable me generate a baseline of airborne micro-plastic pollution in United Kingdom. The impact of Ocean wave and its contribution to airborne micro-plastic pollution, ultimately this will promote the one health perspective.

**5.13.P-Th110 Atmospheric Related Sampling for Microplastics: Developing Rigorous QA/QC Methods**
***Sarah Lavoie-Bernstein***[1,2]*, Razegheh Akhbarizadeh*[1]*, Jasmine Thea Yu*[1]*, Julian Aherne*[3]*, Miriam L. Diamond*[1] *and Liisa Jantunen*[1,2]*, (1) University of Toronto, Canada, (2) Environment and Climate Change Canada, (3) Trent University, Canada*
Microplastics and anthropogenically sourced microparticles (MP-APs), such as synthetic, semi-synthetic and processed natural fibres, are of rising concern globally due to their inherent durability and persistence in the environment. Limited research has been conducted on atmospheric MP-AP abundances, sources, and transport pathways. As a result, representative sampling and quality assurance and quality control (QA/QC) methods are needed to assemble comparable, representative data, especially where collection occurs in remote locations, which favour the use of passive sampling methods.

We developed QA/QC methods that included blanks and standardized reference materials representative of atmospheric particles based on morphology. Here we focus on fibres commonly found in source and remote "sink" locations, e.g., polyester, nylon, and polypropylene. Experimental trials of particle extraction methods and digestion were performed to assess the effects of these processes on MP-APs. Various collection methods used in atmospheric-related sampling were used in this comprehensive study to compare the efficiency of MP-AP collection in ambient air (for particles 5 mm – 10 µm) and analyzed using an Agilent Laser Direct Infrared. These sampling methods included three types of bulk deposition samplers (Nipher Gauge, NILU sampler, bottle and funnel), passive moss bags, polyurethane foam disks, and adhesive strategies, wet precipitation only, as complemented by active high-volume air sampling over the period of one year. Results will be presented.

This research will support monitoring efforts in remote regions, such as the Canadian Arctic, by developing harmonized passive sampling procedures that are needed to establish benchmark abundance and baselines for atmospheric MP-APs. Unlike traditional atmospheric chemical monitoring efforts, which use active high-volume requiring electricity, passive sampling methods require no power and can be deployed by community members, expanding spatial coverage and monitoring. With national and international treaties being developed, these baselines can be used to determine sources, develop mitigation strategies, and assess the effectiveness of regulations.

**5.13.P-Th111 Bioaccumulation of Microplastic Particles in the Eastern Oyster, Crassostrea virginica: One Health Connections**
***Bonnie M. Ertel***[1]*, John E. Weinstein*[2]*, Austin Gray*[3] *and Geoffrey I. Scott*[1]*, (1) University of South Carolina, (2) The Citadel, (3) Virginia Tech*
Microplastics (MP) are ubiquitous in the aquatic environment and have been documented in filter-feeding organisms such as oysters, which are frequently consumed by humans. Along the South Carolina coast (U.S.) where the ecologically and economically important eastern oysters (*Crasssostrea virginica*) form large intertidal reefs, recent surface water surveys found that fibers, fragments, and tire particles represented 43.6%, 30.9%, and 17.7% of the total MP, respectively. The aim of the current study was to characterize the bioaccumulation dynamics of these three particle types in adult eastern oysters. In the laboratory, oysters were exposed to either purple polyethylene fibers, green nylon fragments, or micronized crumb rubber at a concentration of 5000 MP/L. Oysters (n=8 to 10) were sacrificed after 0, 24, 48, and 96 hours of exposure to characterize MP uptake. Following 96 hours of exposure, remaining oysters were transferred to MP-free seawater and sacrificed at 24, 48, and 96 hours (n=8 to 10) to characterize MP depuration. Oysters accumulated significantly longer fibers, smaller fragments, and smaller crumb rubber particles relative to the average sizes to which they were initially exposed. For fibers and fragments, MP levels increased during the uptake phase in a hyperbolic fashion with apparent steady states being achieved at 96 h at 1.61±0.56 particles/g w.w. (mean±SE) and 0.46±0.08

particles/g w.w., respectively. For crumb rubber, levels increased during the uptake phase following a linear relationship with levels after 96-h at 3.62±0.84 particles/g w.w. MP levels in oysters decreased during the 96-h depuration phase by 78.6% for fibers, 80.8% for fragments, and 64.2% for crumb rubber. The present study demonstrates accumulation and depuration of MP and microrubber in eastern oysters are size- and shape-dependent and depuration is an effective method to reduce MP levels. Characterizing the exposure pathways of these pollutants in wildlife and humans will help inform mitigation strategies for a One Health perspective.

**5.13.P-Th112 Microplastics and Other Anthropogenic Particles in Northern Canadian Snow: Occurrence, Distribution, and Characterization by µ-FTIR**

***Jasmine Thea Yu***[1]*, Sarah Bernstein*[1]*, Mary Gamberg*[2]*, Kate Andre*[3]*, Michael Palmer*[4]*, Andrew Platt*[5]*, Cassandra Worthy*[5]*, Miriam L. Diamond*[1] *and Liisa Jantunen*[5]*, (1) University of Toronto, Canada, (2) Gamberg Consulting, Canada, (3) Carcross/Tagish First Nation, Canada, (4) Government of the Northwest Territories, Canada, (5) Environment and Climate Change Canada*

Microplastics, and more broadly, anthropogenic particles, are globally ubiquitous and persistent contaminants. Recent research has shown that some anthropogenic particles (specifically microfibers) can accumulate in the Arctic at levels comparable to those in densely populated locations. Long-range transport via air and/or ocean currents, in addition to local sources, contribute to anthropogenic particle contamination in these remote ecosystems, where they undergo deposition onto land and water. The potential risk is not yet known for Arctic biota and Northern populations who consume country foods. The goal of this study is to investigate the presence of microplastics and other anthropogenic particles in snow collected from Northern Canada, including the high Arctic. Metal scoops were used to collect untouched snow into 10 L cans from Alert (Nunavut), Carcross (Yukon), and lakes in the Northwest Territories from 2020 – 2022. Samples were filtered on polycarbonate filters (47mm) and analyzed by chemical-mapping of entire membrane filters using a focal plane array (FPA) µ-FTIR. Preliminary analysis indicate majority of particles are microfibers. Further results will be discussed. This study will address data gaps and help establish baseline data on microplastics and anthropogenic particle pollution in remote and Arctic environments and improve our understanding of sources, transport mechanisms, and sinks. These baseline concentrations and development of temporal and spatial trends are needed to assess the effectiveness of current and future policy directives.

**5.13.P-Th113 Microplastic Capture by Manufactured Treatment Devices Installed in Storm Sewers**

***Caroline Renee Moore*** *and Barbara Beckingham, College of Charleston*

The environmental risks of microplastics (MPs) (plastics <5 mm in diameter) are actively being researched but the potential for direct and indirect effects in ecosystems is concerning. Microplastics enter the environment from a variety of sources. In urbanized areas, rain events can mobilize a significant load of MPs and transport them through stormwater infrastructure to receiving water bodies. Some municipalities have equipped storm sewers with manufactured treatment devices (MTDs), advanced catch basins that are a stormwater best management practice designed to capture inorganic sediments. As microplastics have a similar size fraction but lower density than sediments targeted by these devices, it is necessary to evaluate their field performance to capture microplastics. We collected sediment grab samples along curb sections of roadways and parking lots, in a set of installed MTDs during scheduled maintenance, and in receiving tidal creeks in Mount Pleasant, South Carolina USA. The objective is to characterize microplastic composition and abundance to determine if microplastics accumulate in MTD sediments and to investigate their dependence on environmental and land-use factors. MTDs were of two types: a multi-chambered, flow-through baffle design and a single-chambered hydrodynamic vortex separator. Preliminary data of the 63-500 µm size fraction from two sites with multi-chambered devices, with one MTD catchment collecting roadway runoff from a residential area and the other collecting runoff from a bridge and parking lot, finds that at both sites MP concentration per dry weight was lowest on the roadways in comparison to MTD and outfall sediments. The residential site contained 18.4 MP/100 g in curbside sediments in comparison to 24.9 MP/100 g in MTD and 118 MP/100 g in outfall sediments. The parking lot site contained 265 MP/100 g in comparison to 625 MP/100 g in MTD and 612

MP/100 g in outfall sediments. This demonstrates that both MTD and outfall sediments can accumulate MPs over time. However, abundance and composition between sites differed. We found fibers to be the most common microplastic at the residential site, accounting for 64% of all microplastics, and fragments at the bridge and parking lot site, accounting for 41% of all microplastics. Forthcoming results from additional sites will provide insight into variation in microplastic loadings in urban stormwater catchments serviced by MTDs and evaluate capture efficiency during storm events.

**5.13.P-Th114 Analysis of lab weathered microplastics using Attenuated Total Reflection Fourier-transform infrared spectroscopy (ATR-FTIR)**

***Shannon Tarby*** *and Erika Holland, California State University, Long Beach*

Microplastics (MPs) are an emerging threat present throughout the globe. They include primary MPs that are intentionally placed in consumer products and secondary MPs that result from the degradation of larger plastics by UV and wave action. Most research has addressed MP toxicity using primary MPs for assessment; however, secondary MPs exhibit different surface chemistry and shapes and are the main form of MPs found in the environment. To determine if we can achieve a similar weathered MP particle compared to those found in the environment I weathered MPs in a rapid weathering device. The weathering device focused UVB and aerating sand on polypropylene (PP) fragments. The extent of weathering was checked at weeks 0,1, 4, 7, 10, 13, and 16 through ATR-FTIR. Here, the FTIR spectrum of polypropylene (PP) was checked for the formation of C=O and double bonds (1640cm$^{-1}$) and hydroxyl groups (3370cm$^{-1}$ - 3240cm$^{-1}$). In a preliminary trial I used the specified area under band (SAUB) Carbonyl index (CI) to identify the extent of weathering as it increases with an increase in exposure time. The SAUB-CI is calculated as: (Area under band 1,850–1,650 cm) / (Area under band 1,500–1,420 cm). An increased SAUB indicates a higher level of degradation. Signs of weathering were evident as early as the 1st week of exposure to UVB and turbulence. Although, after analysis of the weathered MPs it is evident that weathering is not uniform throughout. I will use the weathered MPs in exposures to better address MP toxicity in larval fish. The weathered PP fragments will allow better understanding of how environmentally relevant weathered MPs affect larval fish.

**5.13.P-Th115 Investigating the Relationship Between Microplastics and Beach Invertebrate Communities**

***Maureen Hayden****, Texas A&M University*

The goal of this study is to determine if there is a relationship between beach invertebrate communities and plastic pollution and to identify if marine invertebrates ingest microplastics. Galveston Island State Park, Mustang Island State Park, and Sea Rim State Park were sampled. Collections took place twice per sampling period throughout the year; summer (May-August), fall (September-December), and spring (January-April) from June 2021 through August 2022. Sediment cores were taken to collect invertebrates and sediment samples. Five nearshore transects, each 10 meters apart, were taken from the swash zone using a sediment corer (10 cm diameter, 10 cm h). Invertebrate samples were sorted for each nearshore transect and station. Methods development is underway to determine the best protocol for extracting microplastics from sediment using the Sediment Microplastic Isolation Unit (SMI Unit) and corresponding protocol from Coppock et al 2017. Once the invertebrates are sorted, groups of 10 invertebrates from each taxa (if available) will undergo a tissue digestion assay in 10% potassium hydroxide and be examined for presence/absence of naturally ingested microplastics. Presence/absence data of naturally ingested microplastics from invertebrate specimens and invertebrate community diversity/richness will be compared to microplastics found in sediment core samples from each sample site.

**5.13.P-Th118 Ecological Risk Assessment for Micro- and Nano- Plastics in the Upper San Francisco Estuary**

***Cynthia Kuhn****, Wayne G. Landis and Emma Sharpe, Western Washington University*

This project is conducting an ecological risk assessment for micro- and nano- plastics for the Upper San Francisco Estuary (USFE). Studies in recent years have shown that micro- and nano- plastics are widespread in the environment, and that exposure to them can have toxicological and physiological effects. Because of this, there is an increased interest in understanding how they transport through an ecosystem and what risk they pose to the organisms living there. The ultimate goal is determining what risk they pose to humans. A risk assessment is underway on the San Francisco Bay (SF Bay) itself. This project is extending that risk assessment to the waters in the Delta region, east of San Francisco Bay, containing the North, Central and South deltas, the Sacramento River, Confluence and Suisun Bay. We are using the Bayesian Network Relative Risk Model (BN-RRM) to conduct a risk assessment for chinook salmon, delta smelt and macroinvertebrate community structure. The BN-RRM is a successful framework for regional scale ecological risk assessments of multi-stressor systems, allowing for the creation of a model with predictive capability and adaptive potential as new data become available. This project is relying on plastic particle toxicity data generated by Oregon State University, and site-specific water quality, chemical, and land use data from regional databases. This study is being funded by the National Science Foundation Growing Convergence Research Grant (1935018) program.

**5.13.P-Th119 Microplastics as Vectors of Human Pathogens to Shellfish Bound for Human Consumption**
***Kara J Wiggin**, Jack Gilbert and Sarah Allard, University of California, San Diego*
Microplastics in the marine environment harbor a rich assemblage of microorganisms in their surface biofilms. Recent research suggests the enrichment of potentially pathogenic species, including *Vibrio* spp., on the surface of microplastics in comparison to the surrounding seawater. To determine the potential for *Vibrio parahaemolyticus*, the leading cause of shellfish-related illness in the US, to grow biofilms on plastic, polypropylene surfaces were incubated with *V. parahaemolyticus* for 24h at varying temperatures, and biofilm growth was evaluated using a standardized crystal violet and optical density measurement protocol. High variation in biofilm growth was observed between *V. parahaemolyticus* species, however, all 13 species tested were able to form a biofilm on plastic, depending on temperature, and 11 of the 13 strains demonstrated increased biofilm growth at higher temperatures, with implications for future climate change scenarios. To evaluate the vector capabilities of microplastics, the uptake of *V. parahaemolyticus* in tissue of the Pacific oyster (*Crassostrea gigas*) was quantified after exposure to microplastics with a *V. parahaemolyticus* biofilm or free-floating *V. parahaemolyticus*. Preliminary results reveal a significant increase of *V. parahaemolyticus* in the digestive gland when exposed to microplastics with a *V. parahaemolyticus biofilm* ($p=0.01$) as opposed to free-floating *V. parahaemolyticus,* indicating that microplastics have the potential to magnify the uptake of pathogenic microbes into shellfish bound for human consumption. *C. gigas* is a species of immense economic importance around the world as it encompasses the majority of global oyster aquaculture. The role of microplastics in the transport and uptake of human pathogens of concern into seafood bound for human consumption is a sorely understudied topic and must be explored with further research.

**5.13.P-Th120 Microplastic Research at the U.S. Environmental Protection Agency**
***Kay T. Ho** and Robert M. Burgess, U.S. Environmental Protection Agency*
This presentation covers research performed under the US Environmental Protection Agency's microplastic (MP) program with a focus on research performed at the Atlantic Coastal Environmental Sciences Division in Narragansett, RI. Our program has focused on development of methods for extraction and identification of MP from sediments, citizen scientist methods, and effects research for small MP and nanoplastics. In addition, the presentation will provide a brief summary of MP research performed throughout the Office of Research and Development and the Office of Water. Projects include research performed in collaboration with the Southern California Coastal Water Research Project (SCCWRP), instrumentation and methodology include flow cytometry and laser directed infrared spectroscopy, coral methodology, ASTM methods, and proposed effects research for the next 3-5 years.

**5.13.P-Th121 Microplastics Advanced Research and Innovation Initiative (MARII)**
***John Norman**, American Chemistry Council*
The advancement of a systematic, hypothesis-driven research program is critical to understanding the potential

impact of microplastics on human health and the environment. A global approach linking industry, academia, government is necessary to advance microplastics risk assessment and develop innovative solutions. The Microplastics Advanced Research and Innovation Initiative or 'MARII' is an initiative by the International Council of Chemical Associations (ICCA) to facilitate the global exchange of information on microplastics research, presentations, and current developments. MARII is envisioned as a roundtable to bring scientists from industry, academia, research institutions, governments, and other venues from across the globe to:

- Exchange information on new, ongoing, or recently completed microplastics research and on new emerging methods and technologies; and
- Convene meetings and conferences to collectively discuss, examine, and evaluate current research and initiatives.

In addition to launching MARII, four ICCA member organizations have launched research programs dedicated to addressing many of today's outstanding questions on potential impacts from microplastics. The regional programs of the European Chemical Industry Council (Cefic), Japan Chemical Industry Association (JCIA), American Chemistry Council (ACC), and Plastics Europe (PEu) are designed to develop standardized methodologies and complementary information to fill critical knowledge gaps and needs in microplastics research, within a risk assessment context. Each organization is focused on one area of microplastic research and will coordinate through MARII to ensure the data is compatible across the research programs. This poster will provide the viewer an overview of the research programs developed by Cefic, JCIA, ACC, and PEu. In addition, the MARII program will be reviewed and future directions discussed.

**5.13.V Microplastics in the Environment and Risk Assessment: A One-Health Perspective**

**5.13.V-01 Influences of Wastewater Treatment Plants on Riverine Microplastic Pollution in Japan: Sources-to-Sink Loadings, and Ecological Risks**

***A. H. M. Enamul Kabir** and Masahiko Sekine, Yamaguchi University, Japan*

It is unclear how wastewater treatment plants (WWTPs) in Japan affect the contamination of microplastics in the aquatic environment. This study aimed to explore the loadings of microplastics from the sources-to-sinks (WWTPs-to-rivers-to-marine) around the Yamaguchi prefecture in Japan and assess ecological risks. Surface water samples ($n$=50) from five rivers: Koya, Nishiki, Saba, Shimaji (a tributary of Saba), and Fushino, and effluents ($n$=11) from 11 WWTPs of the catchment of these rivers were studied. All the rivers flow into the Seto Inland Sea (SIS). Filtration, wet peroxidation, density separation, and attenuated reflectance-Fourier transform infrared spectroscopy methods were employed to analyze microplastics. Rivers were ubiquitously polluted with microplastics. WWTPs increased microplastic abundances twofold in their downstream river-stations, suggesting a significant increase, compared to their upstream abundances. The rivers (Koya, Saba, Shimaji, Fushino) impacted by WWTPs, larger populations, and urban areas were more polluted than the less populous and rural Nishiki river. Small microplastics (<500 μm) in size, fibers in shape, polymers— polyethylene, polypropylene, polyethylene terephthalate, vinylon were major both in WWTP effluents and rivers. WWTPs sharply influenced rivers by both abundances and characteristics (shapes-size-polymers) and increased the complexity of microplastic compositions, as a prominent point-source. Microplastic loads from WWTPs-to-rivers (~4.671 billions/day; ~71.8 kg/day), and rivers-to-SIS (~0.13 billions/day/km$^2$; ~7.1 kg/day) presented a substantial emission. The per capita MP emissions to rivers via WWTPs ranged from 0.02 to 6.49 g per day, accounting for approximately 2% of per capita plastic wastes in Japan. An assessment of the ecological risks revealed low to high ecological risks. The WWTPs posed high ecological risks to rivers and built up the pollution hotspots by releasing high number of microplastics, and highly toxic polymers. The observed microplastic compositions from land-sources (WWTPs) to initial (rivers), and final sinks (SIS) are of ecotoxicological concerns. Overall, this study contributes to bridging the knowledge gaps regarding the microplastic sources-to-sinks, ecological risks, and pollution management in Japan and beyond.

**5.13.V-02 Investigating Sea Otter Exposure to Microplastics Using Spraint and Diet Analysis**

***Jennifer Van Brocklin***[1]*, Nicole Duplaix*[1]*, Shawn Larson*[2]*, Taal Levi*[1] *and Susanne M Brander*[1]*, (1) Oregon State University, (2) Seattle Aquarium*

Microplastics and other types of microparticles are present in marine environments throughout the world. Their ingestion has been documented across taxa and shown to have a wide range of deleterious effects. Sea otters play a valuable role as indicators of ecosystem health and several types of their prey have been shown to contain microplastics. We investigated the ingestion of microparticles by sea otters using fecal samples collected in 2019 and 2020 from populations in the Gulf of Alaska. We also analyzed fecal and diet samples from sea otters housed at the Seattle Aquarium for presence and abundance of anthropogenically-sourced microparticles to assess the potential role of trophic transfer in microparticle exposure to these otters. Sample processing included digestion of organic material with potassium hydroxide, a density separation using a 33% hypersaline solution, vacuuming to 14-20 microns, and confirmation of potential plastics with FTIR spectroscopy and Open Specy software. Preliminary results show that potential microplastic particles from wild fecal samples were predominantly fibers (52%) and fragments (26%). Of the subset of microparticles that have been analyzed with FTIR to date, 54% were composed of synthetic material, with another 41% being a material of anthropogenic origin. This analysis indicates that sea otters from vulnerable populations are ingesting anthropogenic debris in the wild. This work is a first step in assessing the impact of microparticle exposure to wild sea otter populations in Alaska and how trophic transfer of microparticles is contributing to this exposure.

**5.13.V-03 Laser-based Identification of Microplastics**

***Louis Tisinger****, Agilent Technologies, Inc.*

Non-destructive detection and identification of microplastics can be a very tedious and time-consuming process, requiring the use of visible microscopy and a very steady hand (for extracting and counting particles). Furthermore, accurate identification is more accurately handled using molecular spectroscopic technologies, such as infrared and Raman, rather physical methods, such as density or melting point. Infrared is the most ubiquitous of the spectroscopic techniques; it has been a mainstay in analytical laboratories for the last six decades. It is especially effective as a tool for quickly identifying microplastics:  almost all organic molecules will have a unique infrared spectrum, a molecular fingerprint that can be used for identifying a plastic particle. When combined with a microscope, microplastic particles as small as a few micrometers can be identified. This presentation will describe an automated laser-based infrared technology for rapid measurement of the requisite physical and spectrometric properties for characterizing microplastic particles.

**5.13.V-04 Microplastics and Plastic Additives in the Snow Deposition From Yukon, Northwest Territories and Nunavut in the Canadian Arctic**

***Alejandra Granados Galvan***[1]*, A.H.M. Enamul Kabir*[1]*, Amila O. De Silva*[2]*, Liisa Jantunen*[2]*, Guillaume Barnouin*[1]*, Alice Guillot*[1]*, Florentine Malaisé*[1]*, Derek Muir*[2]*, Huixiang Xie*[1]*, Youssouf Soubaneh*[1]*, Peter Amarualik*[3] *and Zhe Lu*[1]*, (1) Université du Québec à Rimouski, Canada, (2) Environment and Climate Change Canada, (3) Resolute Bay, Canada*

Plastic pollution is a "cocktail" of contaminants, including physical pieces of plastics as well as a suite of plastic-associated chemicals (e.g., plastic additives) that may be leached into the environment. Microplastics (1 μm – 5 mm) and plastic additives in atmospheric deposition (e.g., snow) are indicators of aerial plastic contamination and are pathways for airborne plastic-related contaminants to enter the soil, aquatic environments, and food webs. Although microplastics have been detected in various matrices in polar environments, our knowledge about the occurrence and fate of microplastics and plastic additives in the atmospheric deposition in the Canadian Arctic is insufficient to effectively assess the levels of plastic contamination and understand their impacts on the polar environments. Therefore, this study aimed to investigate the distribution and characteristics of microplastics (20 μm – 5 mm) and two groups of plastic additives, benzotriazole UV stabilizers (BZT-UVs) and organophosphate esters (OPEs), in snow samples of the Canadian Arctic. Snow samples were collected from Little Fox Lake (Yukon; *n*=6), Yellowknife (Northwest

Territories; *n*=5), Cornwallis Island (Nunavut; *n*=9), and Alert (Nunavut; *n*=15) between February 2020 and May 2021. Filtration, microscopic visualization, and Fourier transform infrared imaging techniques (μ-FTIR) were employed. For microplastics, preliminary results found an average abundance of 27.5 ± 29.4 n/L (mean ± S.D.), ranging from 0.4 to 108.6 n/L. Snow samples from Cornwallis Island (51.4 ± 30.4 n/L) had higher abundance and variation of microplastics, followed by Yellowknife (14.0 ± 5.1 n/L), and Alert (*n*=9, 9.2 ± 11.4 n/L), suggesting higher abundance at the mid and low latitudinal sites in the region, possibly related to local sources. Prevalent shapes were fibers (64%) and fragments (28%), with median lengths of 820 ± 790 μm (mean ± S.D.). Diverse polymers were found, with polyester (43.6%) and polypropylene (21.8%) being the dominant polymers. Measurements and characterization of microplastics in the remaining samples, as well as the analysis of BZT-UVs and OPEs, are currently underway. To this end, the findings may aid in understanding the atmospheric deposition of microplastics and plastic additives in the Canadian Arctic.

**5.13.V-05 Microplastics in the Gulf of Mexico: A Bird's Eye View**

***Elena M.I. Duran***[1]*, Jacquelyn K. Grace*[1]*, Terri J. Maness*[2]*, Mark S. Woodrey*[3] *and Mary Ann Ottinger*[4]*, (1) Texas A&M University, (2) Louisiana Tech University, (3) Mississippi State University, (4) University of Houston*

Microplastic debris is a persistent, ubiquitous, global pollutant in oceans, estuaries, and freshwater systems. The Gulf of Mexico, in particular, has some of the highest reported concentrations of microplastics, globally, and is home to the majority of plastic manufacturers in the United States, as well as being subject to additional plastic discharges from the continents, port areas, tourism activities, river systems, and industrial activities. A comprehensive understanding of the risk microplastics pose to wildlife is critical to the development of scientifically sound mitigation strategies and policy initiatives. In this review, we synthesize existing knowledge of microplastic debris in the Gulf of Mexico and effects on birds, and make recommendations for field and laboratory studies to further this urgent area of research. Although sources of microplastics and observations of microplastic debris in the environment are well-known, further studies are needed to establish the average risk of ingestion for coastal birds, and the hazard that microplastic ingestion poses. The current state of knowledge suggests that microplastics have the potential to be a major ecotoxicological concern for wild birds, especially in areas of high concentration such as the Gulf of Mexico. However, data are currently lacking regarding typical microplastic consumption, uptake of chemicals associated with plastics by avian tissues, and physiological, behavioral, and fitness consequences of microplastic ingestion. Filling these knowledge gaps will be essential to the creation of effective policy actions and widespread mitigation measures to curb this emerging threat to wildlife.

**5.13.V-06 Interaction of Micro and Nanoscale Plastics with Silica Surface in the Presence of Heavy Metals**

***Anika Azme*** *and Mehnaz Shams, Southern Illinois University, Carbondale*

Plastics break down to micro and nanoscale particles and accumulate in the environment due to their inert nature. As these small particles can adsorb other contaminants due to their huge surface area to volume ratio and the presence of diverse functional groups, they are considered as vectors for these contaminants. This study focuses on the interaction of polystyrene (PS) micro and nanoscale plastics with silica surface in presence of heavy metal like copper (Cu) using quartz crystal microbalance (QCM). Deposition and release of materials were investigated on silica surfaces in the presence of increasing ionic strength (IS) of NaCl. Interaction behavior was also observed through batch adsorption process to understand the sorption phenomenon. With increasing IS, both micro and nanoscale PS deposition on silica surface increased due to compression of the electric double layer. No significant deposition was reported in low IS. This could be due to the PS particles following DLVO theory. However, in presence of Cu, micro and nanoscale PS had 5 and 6.5-fold higher deposition respectively under the high IS condition, than in absence of Cu. This was supported by the batch adsorption study revealing that PS had a greater adsorption capacity on quartz sand in the presence of Cu. The presence of positive metal ions can further compress the electrostatic double-layer lowering the energy barrier and causing more PS deposition on the silica surface. Moreover, irrespective of the heavy metal presence, nano PS mass deposition was greater than micro PS. Due to their smaller size, according to DLVO theory nano PS has a lower energy barrier, and causing higher deposition than micro PS. Overall, the results indicate that heavy metals can influence the deposition of micro and nanoscale PS on the silica surface which could affect plastic mobility in the environment. This study would be useful in understanding the interaction mechanisms of PS and other pollutants in the environment for their effective removal.

**5.13.V-07 Microplastic Metrology: Creation and testing of microplastic standard reference materials and their use in harmonizing methods**

***Katherine Shaw***, *Debra Ellisor, Meredith Evans Seeley, Rebecca Pugh, John R. Kucklick and Jennifer Lynch, National Institute of Standards and Technology (NIST)*

Micro- and nanoplastics (MNP) are ubiquitous in the environment and have potential health effects on wildlife and humans, but analytical methods continue to lack consistency and standardization. A commonly requested reference material (RM) is microplastic-sized particles of environmentally-relevant polymers and shapes. Available RMs for MNP research are generally in the millimeter size range and represent only a few polymer types. Smaller size classes are needed to develop methods for the extraction of MNP from various matrices, the determination of additives in MNP, and the identification of MNP polymers through the creation of in-house spectral libraries. To meet this need, the National Institute of Standards and Technology (NIST) is creating a RM with multiple micro-size fractions. We will cryogrind large batches of unused consumer goods composed of five different polymers: polypropylene plastic cups, polyester fabric, nylon anchor line, polyethylene milk jugs, and polystyrene disposable cutlery, using a Palla VM-KT Vibrating Cryomill at ≤ -150 °C in the NIST Cryogenic Reference Material Production Facility. Consumer goods were selected as they represent materials entering the environment from mismanaged waste with relevant plastic additives. The RMs will be useful for example as control materials during the extraction of microplastics from complex matrices. Specifically, the RMs can be applied to all sample types to: 1) estimate the potential polymers and their chemical density, 2) identify potential chemicals to be used for density separation or chemical digestion, 3) determine if preparatory chemicals are compatible with the expected polymers, 4) modify separation apparatus so it is applicable for your sample, and 5) perform spiking recovery experiments. This presentation will update the community on the status of the NIST RM creation and their many utilities. These RMs are crucial for the future of MNP research and risk assessments, and are necessary for a One Health approach to MNP pollution.

**5.13.V-08 Microplastics Exacerbate Disease Virulence in a Common Consumer Salmonid Species**

***Meredith Evans Seeley***[1,2], *Robert Hale*[2] *and Andrew Wargo*[2], *(1) National Institute of Standards and Technology (NIST), (2) Virginia Institute of Marine Science (VIMS) and William & Mary*

Microplastics are an environmentally ubiquitous threat, coexisting with a variety of natural and anthropogenic stressors. In the aquatic environment, for example, pathogens commonly impact multiple trophic levels and human resources. We evaluated the extent to which microplastics mediate the virulence of an important marine disease (Infectious Hematopoietic Necrosis virus, IHNV) in rainbow trout (*O. mykiss)*. Populations of *O. mykiss* are farmed for human consumption across the U.S. North Pacific, Europe and Japan, where IHNV can be highly destructive. To evaluate the hypothesis that microplastics modulate population mortality or incidence of IHNV, we conducted a controlled experiment in which trout were chronically exposed to varying doses of microparticles (0, 0.1, 1 and 10 mg $L^{-1}$) over an 8-week period, with acute IHNV exposure at four weeks. Microplastics used reflected polymers common in aquaculture and wild-caught fisheries: nylon fibers (~10 x 500 µm) used in nets, and ground expanded polystyrene (fragmented to ~20 µm diameter) used in floats. To evaluate if polymers of natural origin (cellulosic plant matter) induce effects, marsh grass (ground to ~20 µm) was included. Mortality was higher when microparticles and IHNV were co-administered than IHNV exposure alone, increasing significantly for nylon fibers at the high concentration and polystyrene at medium concentration. We observed that fish co-exposed to virus and microplastics (particularly nylon microfibers) had a higher viral load and shed than those exposed to virus alone. Importantly, no significant mortality from the presence of any microparticle exposure in the absence of virus co-exposure was observed, underscoring the importance of microplastics as a co-stressor and not just a singular threat. Further, in a follow-up experiment, fish exposed to microfibers chronically prior to IHNV were more susceptible to disease, while microfiber exposure post viral exposure did not augment mortality. We propose that the increase in relative disease virulence arose from increased susceptibility to infection, which may have also enhanced disease transmission between individuals. This work demonstrates that microplastics are an ecologically significant co-stressor for aquatic biota. This may have implications for human diseases transmitted through seafood, and approximate respiratory stressors (particle and virus) for humans – a true One Health concern, warranting further multi-disciplinary research.

**5.13.V-09 Microplastics Exposure: An Emerging Health Threat to the U.S. Military**
***Jennifer Rusiecki*[1]**, *Desmond I. Bannon[2] and Mark Adrian Williams[2], (1) Uniformed Services University, (2) U.S. Army Public Health Center*
The massive growth in global plastic production and use over the past few decades presents a growing threat to human health. Plastic objects degrade into microplastics (MP), defined as plastic particles <5 mm, which are known to be ubiquitous in the environment and thus unavoidable human exposures. Our understanding of human health effects of MP exposures and of smaller nanoplastics (particles < 0.1 µm) exposures is still limited. However, a growing body of literature is reporting measurable levels of MP in human biologic samples, stool, placenta, lung tissue, and most recently human blood. It is becoming increasingly clear that MP in the smaller range (<20µm) translocate from the gut cavity to the lymph and circulatory system, which could lead to systemic exposure and accumulation in tissues. MP exposure may result in particle toxicity via oxidative stress, inflammatory lesions, and increased uptake/translocation. Failure of the immune system to eliminate these synthetic particles may lead to chronic inflammation and thus elevated risk for diseases like cancers, heart disease, diabetes, and bowl diseases. MP may infer additional toxicity via leaching their base constituents (e.g., plasticizers: phthalates, bisphenols), pathogenic organisms, and adsorbed contaminants. There are currently hypotheses about MP/NP exposure and increased risk for obesity, given plausible mechanisms (metabolic disruption) as well as ecological associations between global plastic production and obesity prevalence. There may also be a link with increased prevalence of inflammatory bowel disease, as described in a recent human study. Military populations are likely exposed to MP from similar sources as civilian populations; however, sources of additional MP exposure unique to the military may include: dust/particulate matter during deployment, ingestion of food (e.g., meals ready to eat) and water stored within plastic packaging for lengthy periods in hot environments. Since overweight/obesity has been increasing in the U.S. military in the past few decades, and given the potential for additional MP exposures among military personnel, epidemiologic research and toxicological characterization, focused on military populations is urgently needed to better understand this emerging health threat to the U.S. military.

Disclaimer: The work presented here is the authors' own and does not represent official positions of the Uniformed Services University, Army Public Health Command, or DoD.

**5.13A Microplastics in the Environment and Risk Assessment: A One-Health Perspective**

**5.13A.T-01 Determining Effects of Polystyrene Micro- and Nanoplastic (MNPs) Ingestion on Aedes aegypti and Aedes albopictus Mosquitoes**
***Gabriella H. McConnel*[1]**, *Carla Cristina Edwards[1], Kailash Arole[2], Micah Green[2], Corey Brelsfoard[1] and Jaclyn Canas-Carrell[1], (1) Texas Tech University, (2) Texas A&M University*
Plastic pollution in aquatic and terrestrial ecosystems has become a global issue. While ingestion of large plastics by vertebrate animals has been demonstrated to result in adverse physiological effects, there is little data on the effects of ingestion of microplastics, smaller than 5 µm, on invertebrates. In this study, the effect of microplastic ingestion was examined on two container inhabiting medically important mosquito species, *Aedes*

*albopictus* and *Aedes aegypti*. These mosquitoes are container inhabiting species, exhibiting a preference to deposit their eggs in containers of water (e.g., discarded tires, birdbaths, plastic bottles) rather than natural bodies. Due to ultraviolet degradation and mechanical wear and tear of plastics in the environment as well as long-range environmental transport of airborne nanoplastics, these oviposition locations are likely to harbor secondary microplastics. We hypothesized that ingestion of MNPs will lead to changes in adult emergence rates, survivorship, longevity, hatch rates, and fecundity of mosquitoes. To examine effects on life history traits of *Ae. albopictus* and *Ae. aegypti*, larvae were fed environmentally relevant concentrations of fluorescent tagged polystyrene beads with average diameters of 0.03 µm and 1.0 µm. All treatments were replicated three times and monitored for 40 d post adult emergence. Adult emergence, survivorship, longevity, and fecundity measures were compared to larvae reared in the absence of microplastics. Excreta was collected at different time points post adult emergence and gut dissections performed to observe any physical damage caused by MNPs that might impact mosquito physiology. Although literature has examined sorption of persistent organic pollutants to microplastics, more work is needed to elucidate the relationship between microplastics, pathogens, and vectors of pathogens. Results in this study are discussed in relation to how changes to life history traits as a result of microplastic ingestion could potentially alter vector competence and arboviral transmission.

**5.13A.T-02 Response of a Freshwater Zooplankton Community to Microplastics in Two Large-Scale In-Lake Mesocosm Studies**

***Desiree Langenfeld***[1]*, Kennedy Bucci*[2]*, Cody Veneruzzo*[3]*, Rachel McNamee*[4]*, Chelsea Rochman*[2] *and Michael Paterson*[5]*, (1) University of Manitoba, Canada, (2) University of Toronto, Canada, (3) Lakehead University, Canada, (4) University of Waterloo, Canada, (5) IISD Experimental Lakes Area (IISD-ELA), Canada*

Microplastics (plastic particles < 5mm) are ubiquitous contaminants in aquatic ecosystems, but their ecological effects in freshwater environments are largely unknown. Laboratory studies have shown that microplastics have the potential to negatively impact zooplankton at the individual and population level but the effects for natural zooplankton communities have not yet been studied. We conducted two large-scale in-lake mesocosm studies at the International Institute for Sustainable Development Experiment Lakes Area (IISD-ELA) in northwestern Ontario in 2021 and 2022. In 2021 we added a mixture of three types of common microplastics (polyethylene, polystyrene, and polyethylene terephthalate) to 10m diameter by 2m deep mesocosms in an experimental lake. The concentrations of microplastics were added in an environmentally relevant gradient using a regression design (6, 24, 100, 414, 1710, 7071, 29,240 particles/L) including two control mesocosms with no added microplastics. Preliminary data indicate that microplastics did not have a negative impact on zooplankton abundance or community structure. Five weeks after the addition of microplastics, zooplankton abundance was highest in mesocosms with high microplastic concentrations. In 2022, we used the same mixture of microplastics added at a concentration of 29,240 particles/L to mesocosms like those used in 2021. Using an ANOVA design (n=3 per treatment), we compared effects of microplastics with and without chemical additives against control mesocosms without added microplastics. Results to date will be presented here.

**5.13A.T-03 A Strategy for the Risk Assessment of Micro/nanoplastics for Ecological Systems, Ecosystem Services, Human Health, and Human Well-being**

***Wayne G. Landis****, Cynthia Kuhn and Emma Sharpe, Western Washington University*

Ecological risk assessments are now underway using Bayesian networks and related quantitative tools similar to those being used to estimate risk due to contaminants, water quality parameters, invasive species, emergent disease, climate change, forest fire, and many other stressors. Note that "risk" does not refer to toxicity but to the usual definition in risk assessment where it denotes a probability of affects a specific entity (organism, water quality) and its attribute (pH, mortality, population decline). We have now demonstrated that this set of approaches and tools can be applied to microplastics (specifically tire wear particles) risk assessment. The risk assessment has the same properties as other examples of risk assessments using Bayesian networks as the framework. The outputs are expressed as distributions, uncertainty documentation and sensitivity analysis are straightforward. In our examples we apply the datasets supplied by the Brander and Harper labs that describe toxicity as exposure-response curves with confidence intervals to a variety of species representing specific ecosystem services. In our examples the water quality, co-contaminants, and microplastics exposures are site specific. To date the issues with microplastics such as agglomeration. The main issue has been the difficulties with sampling the complex matrixes associated with marine and estuarine environments for particles at the micro and nano scales. Issues with understanding exposure are typical in the history of ecological and human health risk assessment. However, the risk assessments do point to specific suggestions in order to reduce uncertainty and improve precision: 1) perform exposure-response experiments with sufficient concentrations to generate exposure-response curves 2) use materials that are more representative of those found in the environment , (3) perform experiments and field research to understand the fate and transport of a broad range of compositions and sizes, (4) report whether the toxicity is due to the plastic, other associated toxic materials or a combination, (5) use organisms that represent valued species or ecosystem services, (6) be transparent regarding methods and data and (6) apply statistical tools and graphics based on current best practices and that represent the distribution of the data. This research is based upon work supported by the National Science Foundation Growing Convergence Research Big Idea under Grants #1935028 and #1935018.

**5.13A.T-04 Comparison of Two Procedures for Microplastics Analysis in Sediments Based on an Interlaboratory Exercise**

***Troy Langknecht***[1], *Wenjian Lao*[2], *Charles S. Wong*[2], *Dounia Elkhatib*[1], *Syd Kotar*[2], *Sandra Robinson*[1], *Robert M. Burgess*[1] *and Kay T. Ho*[1], *(1) U.S. Environmental Protection Agency, (2) Southern California Coastal Water Research Project (SCCWRP)*

Microplastics (MP) are globally distributed throughout marine ecosystems and settle into sediments where they may threaten benthic communities; however, methods for quantifying MP in sediments have yet to be standardized. This study compares two methods for quantifying MP in sediments, including isolation, extraction, and identification, and provides recommendations for improving MP analysis for effective monitoring of MP contamination in sediments. Two laboratories compared the performance of two methods, referred to as "core" and "augmentation", for isolating and extracting MP in sediments, and identifying particles with visual microscopy and spectroscopy. Using visual microscopy, the augmentation method yielded mean particle recoveries (78%) significantly greater than the core method (47%) ($p = 0.03$), likely due to the use of separatory funnels. However, spectroscopic identification of recovered particles was much lower at 42 and 54% for the core and augmentation studies, respectively. We suspect the visual identification recoveries are over-estimations of MPs resulting from erroneous identification. This indicates that non-plastic materials persist post-extraction and visual identification alone is not an accurate method to identify MP particles, particularly in complex matrices like sediment. However, both Raman and FTIR spectroscopy proved highly accurate at identifying recovered MP, with 96.7% and 99.8% accuracy, respectively for both methods. Low spectroscopic % recovery of spiked particles indicates that MP recovery from sediments is lower than previously assumed and MP may be more abundant in sediments than current MP analyses suggest. To our knowledge, this is the first multi-laboratory study to quantify complete method performance (isolation, extraction, identification) for sediments, with regards to both capabilities and limitations. This is essential for real-world application as regulatory bodies, such as those identified in California's recent MP legislation, move toward long-term environmental MP monitoring.

**5.13A.T-05 A Potential Vector Into the Food Web: Factors Affecting the Adsorption of Three Microcystin Analogues Onto Six Virgin and Aged Microplastics**

***Diana S. Souza Moura***[1], *Carlos J. Pestana*[1], *Nikoletta Gkoulemani*[2], *John T. S. Irvine*[2], *Jianing Hui*[2], *Christine Edwards*[1] *and Linda A. Lawton*[1], *(1) Robert Gordon University, United Kingdom, (2) University of St. Andrews, United Kingdom*

Water quality is an increasing global environmental concern. An indicator of poor water quality is the presence of cyanobacterial blooms. The hepatotoxin group, microcystins (MC), is the most common group of cyanotoxins reported in freshwater. There are over 200 variants which are often detected as a mixture in the environment. Other pollutants including microplastics are also commonly detected in aquatic systems. There is an increasingly recognized concern that microplastics can act as a vector for micropollutants when they co-exist in the same environment and that they potentially enter the food web. This study evaluated a mixture of three microcystins (MC-LR, -LW, and -LF, 1 µg $mL^{-1}$ each) with six virgin and aged microplastic types using those plastics most commonly reported in the freshwater environment. Particles of polypropylene (PP), polyethylene terephthalate (PET), polystyrene (PS), polyamide (PA), polyethylene (PE), and polyvinyl chloride (PVC) with average particle sizes of 5-45 µm were acquired commercially and artificially aged using the Suntest XLS+ in the laboratory. The virgin and aged microplastics were characterized using Fourier transform infrared spectroscopy, particle size analysis, scanning electron microscope, differential scanning calorimetry, X-ray diffraction, $N_2$ adsorption-desorption surface area analysis, and the zeta potential measurement. The effect of aging microplastics on the adsorption of different microcystin variants was evaluated. This study demonstrated that the aging of the microplastics, the plastic type and the nature of the microcystin are the key factors affecting the adsorption of microcystin on microplastics. The virgin particles of PP, PS, PVC, and PE adsorbed all microcystin variants, whereas virgin PET only adsorbed MC-LW (20%). No adsorption was observed by virgin PA. Among the virgin particles, PP showed the greatest adsorption of the variants, adsorbing from 80% (MC-LR) to 100% (MC-LW/LF). On the other hand, all microcystin variants adsorbed on all aged microplastics, including PA. The largest concentration adsorbed onto virgin and aged microplastics was observed for the more hydrophobic variants, MC-LW, followed by -LF, and finally MC-LR. The results indicate that both virgin and aged microplastics can act as a vector for microcystins in the aquatic environment, with possible implications when they enter the food chain.

**5.13A.T-06 High-accuracy determination of microplastic source using specific profiles of organic/inorganic plastic additives as chemical tracers and estimation for their environmental load**

***Shinnosuke Yamahara**, Thant Zin Tun and Haruhiko Nakata, Kumamoto University, Japan*

In this study, we tried to establish the high-accuracy determination method of microplastic (MP) source based on specific additive profiles in MPs as chemical tracers. Moreover, the environmental load of MPs at the stormwater runoff was estimated in Kumamoto, Japan. At first, over 150 commercially available plastic products were purchased, and the polymer types, organic and inorganic (heavy metals) additives were analyzed by FT-IR, GC-MS, and portable XRF, respectively. Samples of road dust, stormwater, and river sediments were also collected in Kumamoto, and the polymer and additive profiles of MPs in samples were compared with those of commercial plastic products to identify their sources.

As results, 13 polymers, 169 organic additives, and 33 inorganic elements were identified in commercial plastic products, and it showed different profiles among products and their applications. PMMA, PVC, EVA, and black rubber (SBR & NR) were dominant polymers in road dust samples. SBR and NR are well-known as a main component of automobile tire. The organic additive analysis suggested that red-colored PMMA fragments in road dusts contained UV-P (CAS: 2440-22-4), dicyclohexyl phthalate (CAS#: 84-61-7), di-*n*-octyl phthalate (CAS#: 117-84-0). These chemicals are also detected in red paint on the road, suggesting that these PMMA fragments are originated from road paint. Similarly, organic additive profiles in MPs statistically suggested the occurrence of small pieces of braille block, reflector (PMMA), and road marking (PVC) in road dust. Although no organic additives were identified in EVA MPs, the inorganic additive composition implied that road marking and road paint may also become the sources of MPs. These results suggests that plastic additives are suitable chemical tracers of MP sources.

Road-paint-based PMMA fragments and tire-based black rubber (SBR & NR) were also detected in stormwater and river sediment samples. The abundance of MPs in stormwater is 28.5 items/L on average (>100 µm), accounted for 51% of road-paint-based-PMMA and 31% of black rubber (SBR & NR). The environmental load of MPs from this rainwater pipe into small-scale river was estimated to 335~565 million items/year from road paint and 56~464 million items/year from tire wear particles. These values are comparable or greater than those in effluents of Japanese wastewater treatment plants. Considering these observations, road paint and tire are non-negligible sources of MPs in terrestrial area.

**5.13A.T-07 Discussion 1 of 2 - Microplastics in the Environment and Risk Assessment: A One-Health Perspective**

***Mark Surette***[1]*, Mark Adrian Williams*[2]*, Susanne M. Brander*[3] *and Stacey L. Harper*[3]*, (1) WSP USA, (2) U.S. Army Public Health Center, (3) Oregon State University*

**5.13A.T-08 Discussion 2 of 2 - Microplastics in the Environment and Risk Assessment: A One-Health Perspective**

***Mark Surette***[1]*, Mark Adrian Williams*[2]*, Susanne M. Brander*[3] *and Stacey L Harper*[3]*, (1) WSP USA, (2) U.S. Army Public Health Center, (3) Oregon State University*

**5.13B Microplastics in the Environment and Risk Assessment: A One-Health Perspective**

**5.13B.T-01 Effect of Vibrio and Microcystis Co-Exposures on Microplastic Bioaccumulation in the Eastern Oyster, Crassostrea virginica: Implications for Human Dietary Exposures**

***Jessica Wenclawiak***[1]*, John E. Weinstein*[2]*, Peter B. Key*[3]*, Craig Plante*[1] *and Barbara Beckingham*[1]*, (1) College of Charleston, (2) The Citadel, (3) National Oceanic and Atmospheric Administration (NOAA)*

Microplastics are ubiquitous in the aquatic environment, and filter feeders such as the Eastern oyster (*C. virginica*) can accumulate these particles directly from the water column. In addition to microplastics, exposure to pathogenic and toxin-producing bacteria, including *Vibrio* spp. and *Microcystis* spp., can adversely affect bivalves and alter filtration rates. Human shellfish consumers, in turn, can be exposed to microplastics and these bacteria through their diet. The objective of this study was to examine how microplastic co-exposures to *V. vulnificus* and *M. aeruginosa* influence microplastic bioaccumulation in oysters. Oysters were exposed to nylon microfragments and either *V. vulnificus, M. aeruginosa,* or both species and sampled over time up to 96 hours. Following this exposure, remaining oysters were allowed to depurate in clean seawater and sampled over time for up to 96 hours. Microfragments ingested by oysters were quantified and compared between exposure groups, and rate constants for uptake ($k_u$) and depuration ($k_d$) were calculated using a two-compartment kinetic model. Preliminary results revealed that microfragment uptake increased throughout the exposure period, and depuration led to the elimination of microfragments, as expected. Microfragment uptake after 96 hours of exposure was lower in oysters co-exposed to *V. vulnificus* ($\bar{x} = 1.0\pm0.6$ (SD) fragments/g w.w.) or *M. aeruginosa* ($\bar{x} = 1.2\pm0.9$ fragments/g w.w.) than oysters exposed only to microfragments ($\bar{x} = 6.0\pm6.5$ fragments/g w.w.). By studying microplastic bioaccumulation in oysters co-exposed to various stressors, the risk of human dietary exposure to these contaminants can be better estimated.

**5.13B.T-02 Microplastic Accumulation, Excretion, and Effects on the Behavior of Freshwater Organisms-Daphnia magna**

***Jessica Okutsu*** *and Tham C. Hoang, Auburn University*

The recent rapid increase in plastic use and mismanagement of plastic waste have resulted in a plastic pollution problem around the world. Microplastics (1µm - 1mm in size) and nanoplastics (< 1µm in size) are the most concerning because they can last for hundreds of years in the natural environment, accumulate in living organisms, and transfer through the food chain and food web. Although research has been conducted to determine the impact of micro- and nanoplastics on the environment, most research has focused on the toxicity of micro- and nanoplastics on living organisms. Limited research has been performed to evaluate behavioral changes of organisms, which can lead to changing the dynamic of population and community of aquatic ecosystems. This research characterizes the accumulation, excretion, and the effects of microplastics (PE 63–75 µm) on the behavior of *Daphnia magna*. To determine the effects, *D. magna* were exposed to microplastics at different concentrations for over 48 hours and collected over time to determine accumulation and excretion of microplastics. *Daphnia magna* were also monitored for behavior using a NOLDUS behavior monitoring system (EthoVisionXT). The study showed that *D. magna* began ingesting microplastics within the first two hours of exposure. The highest ingestion rate of 9.7 particles per organism were observed at 48 hours of exposure. Microplastic excretion also occurred after ingestion and was dependent on the life stage of organisms and feeding conditions. Results of behavioral monitoring showed that *D. magna* exposed to microplastics spent most of their time at lower depths in the water column, where microplastic abundance is higher. *Daphnia magna* in the control spent time at low and high depths. These results indicate that *D. magna* have changed their feeding niche to the area that has higher microplastic abundance that allow them to ingest more microplastic particles. This would lead to changing population distribution and dynamics of the organisms in natural ecosystem. The ingestion accumulation of microplastics in *D. magna* also reveals a potential consequence of microplastics being transferred through the food chain and food web. Further research should be conducted to characterize trophic level transfers of microplastics into the environment.

**5.13B.T-03 Can We Apply a Site-Specific Ecological Risk Assessment Framework for Microplastics?**
***Rachel Zajac-Fay****, Jason M. Conder and Tina Liu, Geosyntec Consultants, Inc.*
Microplastic toxicity literature for ecological receptors are being published every month. Microplastic state of science is quickly evolving and may lead to the development of regulations, at which point the potential ecological risks posed by microplastics will need to be evaluated in a manner like other regulated chemicals. Although laboratory studies have shown that exposure to microplastics can cause toxic effects in some organisms, these effects still need to be applied to site-specific exposure scenarios to assess risk to ecological receptors at a particular site. Although multiple papers in the past year have proposed microplastic risk assessment frameworks to capture their diverse properties and assess multiple exposure sources for receptors, many data gaps still exist that prevent site-specific ecological risk assessments from being completed. Fundamental data gaps, such as sampling and analysis, quality control, and data reporting, also still exist that contribute to uncertainty in assessing microplastics. Can the proposed frameworks be applied to a site-specific approach, or can a new framework be developed following the EPA's 8-step ecological risk assessment framework for CERCLA sites?  Using the EPA CERCLA paradigm, this presentation will consider how an ecological risk assessment for microplastics can be applied to individual sites by walking through the EPA process. This includes problem formulation (such as selecting appropriate endpoints used in risk decision making); exposure and effects assessments (such as developing direct toxicity values, uptake factors, and wildlife toxicity reference values); and risk characterization (calculating hazard quotients). As microplastics are widespread in the environment, another uncertainty to address is how to factor in an assessment of background concentrations that are unrelated to the site. This presentation will discuss the importance of resolving data gaps so that site-specific ecological risk assessments can be performed and decisions regarding investigations and potential remediation can be made for individual sites.

**5.13B.T-04 International Interlaboratory Intercalibration Study for Microplastics in Environmental Media**
***Charles S. Wong****, Southern California Coastal Water Research Project (SCCWRP)*
Microplastics have a ubiquitous and increasing environmental presence. This increased awareness has prompted recent legislation in California aimed at assessing and mitigating risks of microplastics to humans (Senate Bill 1422) and aquatic ecosystems (Senate Bill 1263). These require the State to develop standardized methodologies to characterize exposure to microplastics in order to compare among measurements, a foundational component needed for assessing exposure, and therefore risks that has long eluded the scientific community.   Accordingly, forty laboratories in 6 counties participated in the intercalibration exercise to address these needs, by receiving and analyzing blind samples with particles of varying polymer types, sizes (1-1000um), colors, and morphologies.  Extraction and analysis were performed on surrogates for drinking water, ambient water, tissue, and porous media, using visual microscopy, Fourier transform infrared spectroscopy, and Raman spectroscopy.  Inter- and intra-laboratory performance and operator experience were assessed using

draft Standard Operating Procedures followed by labs. Processing and measurement method performance was optimized through permutations in SOPs for a subset of labs. Costs associated with training, time, equipment, and consumables were analyzed. Particle recovery was 92±12% (s) in clean water, and decreased dramatically only for the smallest particles (1-20um). Performance was reduced for more complicated matrices that had to be removed, but not to the extent observed for 1-20um particles. Both forms of spectroscopy were accurate and precise, with no significant differences between novice and experienced labs. Training improved precision of measurements considerably. Results from this work has helped to develop the first monitoring program for microplastics in the world, and informed methods and QA/QC for microplastics work worldwide.

**5.13B.T-06 Physical Characterization of Microplastic Contamination in Urban Wet and Dry Deposition**

***Mary Kosuth*** *and Matt Simcik, University of Minnesota*

A seminal study in 2015 quantified microplastic contamination in urban ambient air, sparking intense interest in the atmosphere's capacity to hold and transport synthetic polymer particles. While subsequent investigations of airborne plastic particles at various altitudes, latitudes, and along human population density gradients established the pervasiveness of this global contaminant, sources, sinks, and range of transport remain elusive. In addition, the recent extraction of microplastics from human lung tissue has given this work a sense of urgency. Present research draws on nine months of wet (n = 35) and dry (n = 35) deposition collected from a centrally located rooftop sampler in the third largest metropolitan area in the Midwest. Particle flux, size, and morphology are the three primary metrics used to compare wet and dry deposition and compare two forms of precipitation, rain and snow. This work focuses on specific physical attributes of this durable, low-density pollutant as a means to advance knowledge of its persistence in the atmosphere, the forces that cause settling, and the likelihood of resuspension. Already, preliminary findings reveal distinct trends related to particle size and shape. Monitoring seasonal variation and the meteorological conditions associated with significant fluxes of this contaminant will aid in future risk assessment, as direct inhalation is a recognized pathway of exposure for humans. Results also fit into a larger, interdisciplinary One Health framework, where total abundance and physical characteristics further understanding of this emergent contaminant's interactions with biotic and abiotic components in both natural and engineered environments.

**5.13B.T-07 Panel Discussion 1 of 2 - Microplastics in the Environment and Risk Assessment: A One-Health Perspective**

***Mark Surette**[1], Mark Adrian Williams[2], Susanne M. Brander[3] and Stacey L Harper[3], (1) WSP USA, (2) U.S. Army Public Health Center, (3) Oregon State University*

**5.13B.T-08 Panel Discussion 2 of 2- Microplastics in the Environment and Risk Assessment: A One-Health Perspective**

***Mark Surette**[1], Mark Adrian Williams[2], Susanne M. Brander[3] and Stacey L Harper[3], (1) WSP USA, (2) U.S. Army Public Health Center, (3) Oregon State University*

**5.14.P Opportunities for incorporating ecosystem services approaches into the clean-up and reuse of contaminated sites**

**5.14.P-We176 Using a Decision-Tree Approach to Connect Ecosystem Services Tools and Frameworks to the Risk Assessment Process**

***Matthew C. Harwell**, Tammy A. Newcomer-Johnson, Leah M. Sharpe, Cody Schumacher and Kaitlyn Hines, U.S. Environmental Protection Agency*

There are a range of approaches for connecting ecosystem services (ES) to the ecological risk assessment (ERA) process for contaminated site cleanups. In order of complexity:

- using best professional judgment;

- finding examples from other efforts to apply;
- individualized tool applications;
- using ES-relevant Generic Ecological Assessment Endpoints; and
- developing a systematic, decision-tree approach to crosswalk potentially relevant ES tools and frameworks to the different parts of the ERA process.

This story describes the value-added benefits of using a decision-tree approach and introduces a new tool-navigating portal – specifically developed with risk assessors and contaminated site cleanup practitioners – to identify relevant ES (and ES tools) for a contaminated site ERA effort.

**5.15 Risk and Exposure Tools to Manage Common Metals**

**5.15.T-01 Managing Ambient Metals in Human Health and Ecological Risk Assessment**
***Tamara L. Sorell**, Brown and Caldwell*
Metals can be important drivers of both human health and ecological risk. However, trace elements are naturally occurring and many have nutritional value. Conservative risk tools often do a poor job of differentiating between harmless conditions and chemicals of concern, especially at the early stages of risk evaluation. The majority of elements on EPA's Target Analyte List are considered nutritionally essential and many have daily recommended intakes. All are naturally occurring. However, many of these same elements also have Reference Doses, which may be based on intakes below nutritional levels, or predict risk at background concentrations. There are also inconsistencies in Reference Dose development as to whether dietary sources are considered (such as manganese). Similar issues occur in ecological risk, with low soil screening levels and Toxicity Reference Values often predicting risk at ambient concentrations. For example, a screening level ecological risk for a small mammal several hundred times over the threshold for further evaluation will typically result from inputting a background concentration of aluminum. These imprecise tools often drive unnecessary risk evaluation and may distract from key contamination issues. Suggestions are presented for management of ambient metals in the risk process, including better integration of background, development of toxicity values based on exposures that incorporate environmental bioavailability, and consideration dietary intakes.

**5.15.T-02 Making the World Safe for Shrews: Good Science, Good Policy, or ....?**
***Daniel Smith** and William A Schew, GHD*
Typical methods of ecological risk assessment (ERA) often use shrews as the most sensitive sentinel species for terrestrial systems. These methods routinely find that shrews, *and their ecosystems*, are at "risk" from soil concentrations that are widely occurring and/or at and even below background concentrations The reason shrews almost always "die" is not necessarily related to toxicity or actual potential for ecological effects. Rather, ERAs predict risk due to a combination of the animal's life history and conservative assumptions that sometimes violate biology and common sense. For example, shrews do eat worms, and worms do very efficiently bioaccumulate pollutants from soil. However, worms comprise less than half the shrew diet. In addition, shrews do have extremely high consumption rates per unit biomass, probably the maximum possible for mammals. These extreme consumption rates are accounted for in ERA methods, and the consequent extremely high exposure rates largely drive the assessment of shrew risks. However, basic biology and common sense dictate that shrews also have formidable excretion rates. These prodigious excretion rates would reduce the risks but are not generally considered in assessment of risks. Lastly, ERA methods typically predict that shrew populations*, and their ecosystems*, are impacted because reproduction of individual female shrews is impacted. However, shrews are highly mobile, almost always hungry generalist predators, whose populations are likely controlled by competition for food, not localized reductions in fecundity. The current de facto policy of making the world safe for shrews begs the question of whether they are even an appropriate assessment endpoint. Our analysis dives into these essential but often ignored details, evaluates whether the current approaches adhere to EPA (1998) guidance on ERAs, and identifies potential alternative approaches.

### 5.15.T-03 Evaluating Appropriate Oral Reference Values for Risk Assessment of Copper

***Margaret E. McArdle***[1]*, Alexandra Folcik*[1]*, William L. Goodfellow*[1] *and William J. Adams*[2]*, (1) Exponent, (2) Red Cap Consulting*

Copper, a naturally occurring metal, may act as both an essential nutrient and a toxicant, depending upon dose and exposure. Although various health-based reference values exist for assessing risk from copper from state, federal, and international health authority sources, the U.S. Environmental Protection Agency's (EPA) Integrated Risk Information System (IRIS) database currently lacks an oral reference dose (RfD). Instead, EPA uses environmental media screening concentrations derived from a drinking water action level (i.e., a maximum contaminant level [MCL]) of 1.3 mg/L. In the absence of an oral RfD, EPA and some states have converted the 1.3 mg/L MCL to an RfD of 0.04 mg Cu/kg body weight/day. Other states, as well as national and international agencies, have developed their own allowable doses for risk assessment, allowable dietary intake, or limits for copper in drinking water, which range greatly. Therefore, a recommended oral value for copper could simplify and streamline risk assessments of copper throughout the U.S. To identify appropriate oral reference values for risk assessment of copper, we evaluated (1) authoritative reviews on copper, (2) copper oral reference values and action levels used by several individual U.S. states and (3) key studies from the scientific literature on copper toxicity, including various human health endpoints, essentiality, and exposure, including background exposure. Our evaluation found that an oral RfD of 0.04 mg Cu/kg body weight/day (supported by categorical regression) is protective of acute or chronic toxicity in adults and children and is supported by available health-based evidence for copper.

### 5.15.T-04 Short-Term Environmental Inhalation Toxicity Criteria for Airborne Manganese Protective of Neurological and Respiratory Effects for use in Air Toxics Risk Assessment

***Camarie S. Perry****, Ann Verwiel, Todor Antonijevic, Stephanie Vivanco and Deborah M. Proctor, ToxStrategies, Inc.*

Manganese (Mn) is an essential element; the absorption and excretion of which is controlled by homeostasis. As such, derivation of toxicity criteria should consider non-linear toxicokinetics. Mn is also well recognized to cause central nervous system (CNS) effects and respiratory distress in association with high concentration chronic and sub-chronic airborne exposure in some occupations. The need for acute environmental Mn guidelines for regulation of air toxics has recently developed, but only limited data exist to develop a short-term health-based guideline. We reviewed US state, federal, and international health-based environmental inhalation toxicity criteria, as well as occupational exposure limits, and their toxicological bases. Next, a literature search of relevant publications from 2005 though 2022 was performed to capture newly published studies. The study deemed most appropriate to provide the basis for a 24-hour exposure guideline was an inhalation study performed in monkeys exposed 5 hours per day, 5 days/week, for 3 weeks in which a lowest observed adverse effect level (LOAEL) of 1.5 mg/m$^3$ was reported for inflammatory airway changes (e.g., mild bronchiolitis, alveolar duct inflammation). This study was also used by the Texas Commission on Environmental Quality (TCEQ) as a point of departure (POD) for establishing its short-term screening values for Mn. To this POD, we applied a cumulative uncertainty factor of 300 accounting for interspecies, intraspecies, and LOAEL to no observed adverse effect level (NOAEL) extrapolation, resulting in a conservative short-term guideline of 0.005 mg/m$^3$. To assess the potential for CNS effects at this exposure guideline, previously published Mn physiologically based pharmacokinetic (PBPK) models for children and adults were used to predict levels of Mn in the brain (i.e., globus pallidus) for 24-hour exposure at 0.005 mg/m$^3$. The PBPK model predictions for Mn in the globus pallidus were not significantly increased compared to diet alone and background Mn in ambient air, and well below NOAELs of 0.7–0.9 µg/g for neurological effects reported in the literature from human and primate studies. Using refined risk assessment methods, this study supports a short-term guideline for environmental exposures of 0.005 mg/m$^3$ for use in air toxics risk assessment, including welding-fume particulates, which is equal to the TCEQ guideline and 17-times higher than that set by the Oregon Department of Environmental Quality.

**5.15.T-05 Metal pollution in aquatic ecosystems become a cause for concern: A case study of the Umgeni River system, South Africa**

***Jeffrey Lebepe***[1]*, Ashish Misra*[2]*, S'phosakhe Mdluli*[2]*, Mandlenkosi Nkala*[2]*, Sanelisiwe S.B, Hlatshwayo*[2] *and Ajay Bissessur*[2]*, (1) Sefako Makgatho Health Sciences University, South Africa, (2) University of KwaZulu-Natal, South Africa*

Rivers are among the most threatened ecosystems, globally. In South Africa, the Umgeni River is one of the most polluted systems in KwaZulu-Natal province. The river drains a catchment characterized by extensive agriculture, wastewater works, and metallurgic industries. The river is home to over 40 fish species and it runs through poor communities which opt for fish to supplement their protein needs. The present study assessed metal pollution in Nagle and Inanda dams, and the edibility of inhabitant fish. Samplings were carried out during low and high flows in 2019 and 2020. Surface water and sediment were sampled using acid-pretreated water bottles. Three preferred fish species, *Clarias gariepinus*, *Oreochromis mossambicus*, and *Coptodon rendalli* were sampled using gillnets and electro-shocker. Fish lengths and weights were measured, and liver, gill, and muscle tissues were harvested. Tissues were digested using aqua-regia and hydrogen peroxide. The condition factor and hepatosomatic index exhibited normal ranges for both populations. Most metals were below the detection limit in the water column, however, substantially high concentrations were observed in sediment. Dispersion showed no clear separation for the liver, gill, and muscle for each species between the two dams ($p>0.05$), whereas a clear separation was observed between species in each dam ($p<0.05$). Moreover, the concentrations showed no significant difference for each species between the two dams, however, a significant difference was observed between species in each dam (MANOVA, $p<0.001$). Poor to moderate relationships were observed for fish lengths and metal concentration in all tissues for the three populations. The THQs exceeding 1 were observed for the concentration of As, Cr, Sb, and Pb in the muscle of all species. The *Clarias gariepinus* showed a relatively higher THQ followed by *O. mossambicus* and *C. rendalli*, respectively. These findings reveal that the consumption of fish from the contaminated Umgeni River poses non-carcinogenic human health risks and recommend urgent intervention by responsible authorities to mitigate pollution levels to ensure the safety of fish consumers.

**5.15.T-06 Baseline Ecological Risk Assessment of a Metals-Impacted Pond: Using Site Specific Information to Focus Remediation Goals**

***Lisa M. McIntosh*** *and Kyle Apigian, Woodard & Curran*

Conducting baseline ecological risk assessments (BERA) can be costly and labor-intensive, but BERAs often provide comprehensive, site-specific information that ultimately may result in reduced cleanup costs. Past disposal operations at a former enamel coatings facility, which included a series of lagoons to which wastes discharged, resulted in metals contamination in soil, groundwater, sediment, and surface water in a freshwater pond. Historical sampling suggested potential ecological impacts across the entire pond. We used BERA to demonstrate impacts, previously thought to extend across the entire 120-acre pond, were limited to a small cove within the pond, thus allowing us to focus further response actions to approximately one acre. The multi-step BERA investigation to fill in data gaps on the nature and extent of metals impacts included collection of chemical data for surface water, sediment, groundwater, invertebrate and plant tissue samples; bioaccumulation and toxicity testing; and wildlife exposure modeling/food chain analysis. Ecological risk, mainly characterized by toxicity to benthic organisms, was identified to be limited to only a small portion of the pond. Logistic regression was used to evaluate the statistical relationship between metals concentrations in sediment and benthic invertebrate toxicity, taking into consideration results from reference locations. Total metals concentrations in sediment (adjusted for percent organic carbon) were found to be significantly correlated with reduced survival and reproduction of benthic test organisms. Using these correlations, we derived sediment cleanup levels for the pond. Application of the site-specific BERA resulted in significant reduction of the initial estimated impacted area and ultimately will focus remediation, reduce cleanup costs, and minimize impacts to sensitive habitats that may otherwise have occurred through intrusive remediation activities.

**5.15.T-07 Background Metals in Risk Assessment and Risk Management, It Ain't That Hard**
*Francis Ramacciotti, William A. Schew,* ***Tamara House-Knight*** *and Sam Townsend, GHD*
In environmental risk assessment we deal with hundreds of uncertainties and unknowns/little knowns in every assessment. Why do background metals continue to be something that requires substantial justification? RAGS Part A (1989) recognizes background and OSWER Directive 9355.0-30 (1991) recognizes the concept of "site risk." Nearly every Feasibility Study, Corrective Measures Plan, etc., separates site-related sources of contamination from background sources. Yet, in the risk assessment, practitioners are often asked to spend significant amounts of time evaluating potential exposure to metals concentrations in the environment that are not related to the site and are by any other name "background." When practitioners are "allowed" to account for background quantitatively, they often have to provide quantitative, qualitative, and statistical justification that metals are background (even when/where the precedent has been long established).

This work documents and summarizes some methods that have been historically used by the authors to "sufficiently" document that metals concentrations at a site are background. The methods include evaluation relative to published studies, using EPA approaches to calculate background groundwater concentrations from soil concentrations, using EPA statistical tools, direct (and complicated) evaluations of relative datasets, etc. This presentation is intended to serve as a practical guide for regulators and practitioners to see solutions that have been accepted. The audience can hopefully conclude that with background metals, Occam's Razor is superior to substantial investment of effort that likely does not change the conclusion or improve decision making.

**5.15.T-08 Discussion - Risk and Exposure Tools to Manage Common Metals**
***Tamara L. Sorell****, Brown and Caldwell*

**5.15.P Risk and Exposure Tools to Manage Common Metals**

**5.15.P-Th126 Synergistic role of copper and cadmium in multi-metal toxicity and oxidative stress**
***Sam Li****, NUS, Singapore*
Since industrial wastewaters frequently contain high quantities of metal mixtures, there is a need to determine how metal combinations may have synergistic or antagonistic impacts on aquatic life. To study the effects of multi-metal systems (Cu, Cd, Pb) on aquatic ecosystems, we used a combination of inductively coupled plasma mass spectrometry (ICP-MS)-based metallomics and nuclear magnetic resonance spectroscopy (NMR)-based metabolomics. Metallomic and metabolomic results revealed that Cu and Cd have synergistic effects on growth suppression and toxicity. While Pb had less significant influence on the metabolome. Cu-induced oxidative stress resulted in a significant drop in key metabolites and their precursors. Total elemental analysis also revealed a considerable decrease in K and an increase in Na, Mg, Zn, and Mn contents. In this study, Cu was found to have higher ecotoxicity than Cd or Pb, and the combination of Cu and Cd could cause a high synergistic effect on oxidative stress induction.

**5.15.P-Th122 Heavy metal(loid) exposure and occurrences of respiratory outcomes, lipid peroxidation and DNA damage in residents of a Ghanaian industrial/commercial city**
***Nesta Bortey-Sam***[1]*, Osei Akoto*[2]*, Yoshinori Ikenaka*[3]*, Shouta M.M. Nakayama*[3]*, Elvis Baidoo*[2]*, Kwadwo Ansong Asante*[4] *and Mayumi Ishizuka*[3]*, (1) University of Pittsburgh, (2) Kwame Nkrumah University of Science and Technology, Ghana, (3) Hokkaido University, Japan, (4) Council for Scientific & Industrial Research, Ghana*
Metal/loid contamination resulting from industrialization is of major concern due to the reported health risks. Previous studies of metal contamination in environmental matrices, including tissues of rats, have been widely studied in Kumasi, Ghana, and despite the potential risks to residents, there is limited study linking exposures to adverse health outcomes. This study therefore explores any relationship between metal/loid exposure and respiratory diseases, lipid peroxidation and/or DNA damage occurrence among Kumasi residents. From the

metal urinalysis, total As was higher in >80% of participants compared to the reference site. Urinary metals, malondialdehyde (MDA), and 8-hydroxy-2-deoxy-guanosine (8-OHdG) were higher in participants living in urban sites vs the control site. The study showed that urinary Cd and MDA were highest in the elderly and the young, respectively, while significantly higher urinary Co, As and Cd were detected in female participants. Arsenic exposure was significantly associated with asthma (OR = 2.76; CI: 1.11-6.83) and tachycardia (OR = 3.93; CI: 1.01-15.4). The study showed a significant association between urinary metal/loids and MDA and 8-OHdG, pointing to the likelihood of lipid peroxidation and/or DNA damage among the participants.

**5.15.P-Th123 Probabilistic Health Risk Assessment for Residential Exposures to Metals in Electric Arc Furnace (EAF) Steel Slag**

***Deborah M. Proctor****, Liz Mittal, Stephanie Vivanco and Todor Antonijevic, ToxStrategies, Inc.*

EAF slag is a co-product of steel production and is used primarily for construction purposes, including landscape aggregate and cover for unpaved rural roads. Some applications of EAF slag may result in residential exposures by direct contact and inhalation of airborne dust. To evaluate the potential health risks, an EAF slag characterization program was conducted to measure concentrations of metals in slag, leaching potential (including oral bioaccessibility [BA] by EPA Method 1340), and analysis of mineralogy. EAF slag is an alkaline mineral matrix. Comparing metal concentrations in EAF slag to residential Regional Screening Levels, antimony, arsenic (As), hexavalent chromium (CrVI), iron, vanadium, and manganese (Mn) were identified as constituents of interest, although As levels in slag are consistent with naturally occurring As in soil. Using a probabilistic risk assessment (PRA) approach, exposures specific to residential EAF slag applications for driveways/landscape and for residents near unpaved roads were assessed for children and adults. Varying assumptions regarding particulate emissions, time spent at home, and time spent outdoors were considered, with exposure parameters from EPA guidance and measures of EAF slag-specific oral BA. For Mn, measures of relative bioavailability (RBA) from a recently conducted *in vivo* rat RBA study of EAF slag will be included. The PRA predicted the distribution of dose, and the increased cancer risks and hazard indices (HIs) for the $50^{th}$ and $90^{th}$ percentiles of exposure were determined. For the residential roadside scenario, cancer risk and HIs were < 1E-6 and 1, respectively. For the residential driveway/landscape scenario, cancer risks were 2E-6, assuming 100% oral BA for CrVI, and the HI was 1 using EPA's most conservative Mn reference dose, and BA as an estimate for RBA. To further assess the potential for Mn neurotoxicity, a published physiologically based pharmacokinetic (PBPK) model was used to predict levels of Mn in the brain (i.e., globus pallidus) for children and adults. The PBPK model predictions for Mn in the globus pallidus were slightly increased at the $90^{th}$ percentile (0.51–0.56 μg/g) compared to diet alone, but were well below NOAELs of 0.7–0.9 μg/g for neurological effects reported from human and primate studies. Overall, using refined risk assessment methods including PBPK modeling, the PRA found that application of EAF slag in residential areas does not pose a health hazard.

**5.15.P-Th125 Considerations for Determining Ecological Guideline Values for Boron and Other Essential Elements**

***Austin Wilkes****, Samuel Parker, Gary R. Long and Nigel Goulding, EHS Support LLC*

There is currently a lack of consensus on appropriate protective guideline values for boron in the aquatic environment. Boron is toxic at high concentrations but is also an essential micronutrient required by organisms across multiple phylogenetic kingdoms. Essential elements like boron require special considerations at the study-level and regulatory-level to accurately characterize ecotoxicity and derive appropriate protective guideline values. In the present study, we examine how essential element deficiency and toxicity complicates commonly used approaches to derive protective values and make recommendations on more robust approaches for boron. This work consisted of a meta-analysis of existing literature on boron ecotoxicity, deficiency, essentiality, and regulatory guidelines for protection of ecosystems. Toxicity testing for essential elements requires special considerations at the study level to obtain meaningful results. Because deleterious effects are expected at both low concentrations and high concentrations, identification of the optimal concentration range is a critical first step in toxicity testing in order to set an appropriate test control and ultimately obtain meaningful results. At the regulatory level, protective guideline values are often derived using species sensitivity distributions (SSDs), which implicitly assume that lower concentrations will always result in increased species protection. Since this assumption does not hold true for essential elements, guideline values derived using SSDs may be protective of a lower percentage of species than purported. This study advances our understanding of the challenges associated with establishing meaningful protective guideline values for essential elements and promotes more holistic consideration to both toxicity and deficiency in risk-based decisions for the aquatic environment.

**5.16.P Risk assessment for Low Risk / Reduced Risk Pesticides**

**5.16.P-Th127 Emerging Safeners and Herbicides Induced Toxicities in Daphnia magna and Zebrafish Embryos**

***Oluwabunmi Femi-Oloye*** *and John Giesy, University of Saskatchewan, Canada*

Plant safening chemicals known as safeners have been found in water bodies and are implicated in causing some adverse effects in non-target species of the aquatic environment. Safeners' environmental occurrence, mode of action and effect on non-target species are poorly understood because they are classified as inert. Emerging safeners, cyprosulfamide (CYP) and Mefenpyr-diethyl (MEF) and their co-herbicides; Isoxaflutole (ISO) and Fenoxaprop-p-ethyl (FEN), were used in this study. *Daphnia magna* and Zebrafish (*Danio rerio)* embryos were exposed to varying concentrations of these safeners and herbicides, singly and mixed, following the OECD method with slight modifications to assess effects on some endpoints, including mortality and reproduction. The chronic exposures were done using static-renewal methods for 21 days. A single *D. magna* of less than 24hrs of age was placed in each treatment jar, and there were ten true replicates for each treatment. Also, approximately two hours post-fertilization (2hpf), embryos were introduced into each well of a 24-well plate in a temperature-controlled chamber, and we experimented in triplicate. Concentrations used include 0. 3,6,9,12 and 15 mg/L for MEF; 0, 5, 20, 40 and 90 mg/L for CYP on daphnids, 0, 0.1, 0.5, 1.5, 3 and 5 mg/L for MEF and 0, 0.1 and 1 mg/L were used for mixtures of both safeners and their co-herbicides on zebrafish embryos. Results indicated that these chemicals did not affect the embryos' heart rate and hatching rate across all concentrations at 96 hours. However, with exposure of daphnids to MEF and CYP at lower concentrations, the number of neonates increased. In contrast, an increase in concentration leads to a decrease in neonates' numbers - a condition known as hormesis. Hormesis points toward the stress that could result from exposure to the chemicals. A 100% mortality of zebrafish embryos was recorded with 3 and 6 mg/L concentrations of MEF, while the mortality rate increased with the MEF mixture using the static renewal method. This study shows that safeners and herbicides can cause perturbations that may impact freshwater invertebrates and vertebrates; however, the mechanism of action inducing such response should be studied. This study can help chemical regulators with effective regulatory policies and chemical classification.

**5.16.P-Th128 Does Pesticide Use Influence Phenology and Fitness of California Birds? A Study Using Citizen Science Data**

***Yuzhong Zhang*** *and Andrea Bonisoli Alquati, California State Polytechnic University, Pomona*

The invention of pesticides gave the agricultural industry a chance to match up against the fast-growing food demand. However, widespread use of agricultural pesticides has proven toxic also to non-target organisms and their ecosystems. Birds’ relatively high positions in the trophic web of agroecosystems expose them to the threat. Research has shown that farmland birds are in a steeper decline than birds from other ecosystems. Although such decline most likely results from the synergism of several factors, many studies suggest pesticides as one of the potential causes. This study uses geographic information systems (GIS) to investigate whether agricultural pesticides are associated with negative impacts on birds’ fitness, by analyzing how pesticides use, in space and time, predicts the abundance, phenology, and fitness of birds. To this aim, we will leverage large citizen science datasets about bird abundance and breeding phenology, paired with pesticide use data from the

California Department of Pesticide Regulation. Priority will be given to widely used pesticides with demonstrated toxicity to birds in laboratory or field studies. These will include chlorpyrifos, imidacloprid, clothianidin, and fipronil, which have been shown to impair thermoregulation, orientation ability, food consumption, and energy expenditure, leading to negative impacts on their fitness. In this study, agricultural areas with high availability of citizen science data and intense pesticide use will be compared to ecologically similar areas with lower pesticide use. To account for interspecific variation in sensitivity to stressors we will build species-specific multiple linear regression models for each species. We hypothesize that higher pesticide use will be associated with lower local abundance of birds, later breeding dates, and lower clutch size and fledging success. Habitat conversion and loss and climate change may also be reasons for the decline of avian populations. To untangle exposure to pesticides from other stressors, we will include a suite of bioclimatic variables, and factors and covariates related to anthropogenic activity, such as crop type, human population density, and impervious surface. Information-theoretic approaches will help select the most informative models explaining bird phenology and fitness. Overall, this study will contribute to a better understanding of the true ecological costs associated with pesticide use in California.

### 5.16.P-Th129 Determining Toxicity of the not so "Inert Ingredients" in Pesticides to Adult Worker Honey Bees

***Brandon Patrick Shannon**, Emily Walker and Reed Johnson, The Ohio State University*

The principal functioning agents that make up spray adjuvants and serve as “inert ingredients” in formulated pesticides are a diverse group of agrochemicals that are added to pesticides to improve the function of spray application through increased leaf sticking, spreading, and penetration. The significant honey bee colony losses that have been reported during and after almond pollination in California may be related to exposure to these compounds during almond pollination. The aim of this research was to determine if formulated adjuvants, individual principal functioning agents, and field-relevant mixtures of formulated adjuvants or individual principal functioning agents in combination with pesticides applied during almond bloom can cause increased mortality in adult worker honey bees exposed to simulated spray applications. This study established the acute toxicity, expressed as $LC_{50}$, of different adjuvants and adjuvant-pesticide tank-mix combinations. Spray application was performed using a Potter Spray Tower on 3-day-old adult worker honey bees. Tested adjuvants included Dyne-Amic, Kinetic, Surf-90, Induce, Cohere, Liberate, Activator 90, Nu Film P, Li-700, Choice, Latron B, and Attach; tested principal functioning agents included methyl esters of fatty acids, alcohol ethoxylates, fatty acid ethoxylates, organic polymers, and organosilicone polymers; tested fungicides included Pristine, Tilt, Vanguard, and Luna Sensation; and tested insecticides included Altacor and Intrepid. Results show that some adjuvants and some principal functioning agents applied alone at field relevant concentrations can cause adult bee mortality. Results also indicate a trend in increased toxicity of some adjuvants and some principal functioning agents when applied as a tank mix with formulated pesticides.

## 5.17 Soil Contaminants: Fate, Bioavailability, Environmental Toxicology in Ecological and Human Health Risk Assessment

### 5.17.T-01 Application of Springtails and Oribatid Mites as Bioindicators of Petroleum Hydrocarbon Soil Toxicity

***Prama Roy**[1], Barb A. Zeeb[2] and Allison Rutter[1], (1) Queen's University, Canada, (2) Royal Military College, Canada*

Soil invertebrates are valuable indicators of soil ecosystem health and nutrient cycling as they feed on decaying materials, excrement, algae and fungi in their habitats. Over the last 30 years, the use of springtails (subclass: Collembola) and oribatid mites (order: Oribatida) have become popular in ecotoxicological studies due to their rapid and high sensitivity to numerous environmental conditions. Standardized toxicity tests using soil invertebrates may aid in the derivation of site-specific environmental guidelines that address the physiological impact of soil contamination on native terrestrial species. Canadian standardized survival and reproduction tests are underway using two age-synchronized springtails and an oribatid mite species that were placed in petroleum hydrocarbon contaminated soil (>16,000 ppm) from a highly contaminated site in northern Ontario. The soil is primarily contaminated with semi-volatile Fraction 2 (10,300 ppm) and non-volatile Fraction 3 (4,960 ppm) hydrocarbons. In particular, these F2 polycyclic aromatic hydrocarbons and alkylated derivatives were found in the highest concentrations: i) phenanthrene, ii) fluorene, and iii) naphthalene. Contaminated soils were diluted with 0%, 25%, 50%, 75%, and 100% relative volumes of background soil (<100 ppm) collected from the site vicinity to create dose-response curves and identify the 50% lethal concentrations ($LC_{50}$) and 25% reproductive inhibitory concentrations ($IC_{25}$) for the test species. This paper will report on the test methodologies and performance of each species during the survival and reproduction tests currently being carried out.

**5.17.T-02 Linking Reproductive Toxicity to Gene Expression in a Soil Invertebrate, Folsomia candida, Exposed to Petroleum Hydrocarbons**

***Adrian Pang***[1]*, Allison Rutter*[1] *and Barb A. Zeeb*[2]*, (1) Queen's University, Canada, (2) Royal Military College, Canada*

Soil invertebrates play a crucial role in the nutrient cycle and are often more sensitive to contaminants than plant species, making them valuable predictors of soil quality. Many standardized test methods exist to investigate the effects of contaminants on soil invertebrates at the individual level, but often take several weeks to complete. With advancements in next-generation sequencing, transcriptomics can be used to identify molecular markers in order to predict the toxicity of contaminated soils more rapidly. However, the association between gene expression and toxic effects at the individual level are still not fully understood for many species. The present study aims to establish a link between the gene expression of a soil invertebrate species, *Folsomia candida*, to its reproductive EC values, when exposed to petroleum hydrocarbon-spiked soils. The EC50 value for *F. candida* reproduction was determined following biological test methods published by Environment Canada. Gene expression rates of *F. candida* exposed to the ½ EC50, EC50, and control soil, were compared to determine differentially expressed genes. This study improves the understanding of petroleum hydrocarbon mode of action in *F. candida*. In addition, biomarkers linked to toxic effects on reproduction are presented, which may be valuable and rapid alternatives to traditional toxicity tests.

**5.17.T-03 Leaching: A Solution to Counter Ion Interference in Phytotoxicity Assessments for Rare Earth Elements Added to Soil**

*Sharareh Dehghani, Luba Vasiluk and* ***Beverley A. Hale****, University of Guelph, Canada*

Concern for rare earth element (REE) toxicity to plants is rising due to the worldwide increase in their use for consumer goods, and thus their extraction through mining; they also are added to soils incidentally or intentionally through agriculture. To determine toxicity thresholds for REEs and plants, dose-response studies are required, which use REE-amended soils. The form of REE added to soils can be the nitrate salt, thus the observed phytotoxicity is a combination of responses to REEs and nitrate. This study distinguished the responses of durum wheat (*Triticum durum*, var. 'Kyle') and tomato (*Solanum lycopersicum*, var. 'Bonnie's Best') to three REEs (cerium (Ce), neodymium (Nd), and europium (Eu)) from their responses to the nitrate component of their salts under greenhouse conditions. There were two approaches: nitrate content of the soil was equalized by adding $KNO_3$ as a counterion; and soils were leached after amendment to return soil electrical conductivity (EC) to control levels. For both conditions, toxicity thresholds were determined through growth studies, and soil pH and REE bioaccessibility measured. In some cases, nitrate predominantly drove toxicity thresholds in unleached soils. Leaching the REE-amended soil was used to deplete counterion concentration; it successfully removed excess nitrate and decreased soil salinity with no significant loss in REEs. Most endpoints determined for leached REE-amended soils had higher toxicity thresholds, confirming nitrate or salinity toxicity in the unleached soils; this was more intense for Eu than for Ce and Nd. There were only four cases where there were $EC_{50}$ for both unleached and leached: all were for tomato, for root and shoot length response to Ce, and for shoot length and biomass response to Nd. The ratio of leached $EC_{50}$/unleached $EC_{50}$ was 3.9 and 4.1 for shoot length response to Ce and Nd, respectively, 4.0 for root length response to Ce and 3.8 for shoot dry mass response to Nd. The relationship between accumulation of these REEs in shoots were consistent with the variations in bioaccessible soil REEs; however, REE bioaccessibility was not always following changes in soil pH values, which varied as a result of the plant's utilization of nitrate.

**5.17.T-04 Development of Site-Specific Soil to Earthworm Bioaccumulation Model for Polychlorinated Biphenyls**

***Holly McChesney****, Suzy Walls, Nancy Bonnevie, Adam Ayers and Paul Anderson, Arcadis U.S., Inc.*

When evaluating potential ecological risks associated with polychlorinated biphenyls (PCBs) in soil, estimating the potential bioaccumulation by invertebrates such as earthworms is a primary uncertainty. Because of biomagnification, estimates of PCB concentrations in earthworm and other invertebrate tissues affect predicted exposure and potential risk associated with higher trophic level receptors. Therefore, understanding uptake into these organisms can greatly increase the predictive ability of ecological risk assessments and allows for more refined estimates of potential risk. Despite the importance of this pathway, relatively few studies exist from which reliable bioaccumulation factors (BAFs) can be derived. Commonly used literature-based BAFs are based on limited laboratory and field datasets and provide highly variable estimates of PCB uptake to earthworms. To address this uncertainty, co-located soil and earthworm tissue samples were collected from 20 locations within the Upper Hudson River floodplain and analyzed for PCBs with the objective of refining the understanding of PCB accumulation in earthworms from floodplain soils. A soil-to-earthworm bioaccumulation model was developed using these data and compared to soil-to-earthworm BAFs and bioaccumulation data available in the literature. The site-specific bioaccumulation model better predicted observed earthworm concentrations than any of the literature-based models or BAFs. The results emphasize the importance of site-specific factors that can significantly alter uptake of PCBs such as congener composition, weathering, and other abiotic or biotic factors.

**5.17.T-05 Determining the Bioavailability and Leaching Potential of Arsenic in Wildfire Ash and Debris**

***Erik Naylor****, Maul Foster & Alongi, Inc.*

The 2020 Oregon wildfires burned millions of trees and destroyed thousands of homes and buildings throughout the state. Ash and debris from destroyed residential structures is extremely hazardous and may contain metals and asbestos. Disaster declaration made funding available through the Federal Emergency Management Agency and the Oregon Department of Transportation was selected as the state agency to administer the cleanup. Property owners could enroll in the program to have state contractors cleanup their property for free. After ash and debris removal, confirmation soil samples were collected at each property and measured for metals to determine if removal was successful. A combination of risk-based and natural background concentrations was used for screening levels to determine if impacts related to wildfire ash and debris were successfully removed at each property. The natural geology in Oregon causes high levels of arsenic in soils relative to typical risk-based screening levels. Determining whether arsenic concentrations in soils were due to wildfire impacts, historical contamination, or naturally occurring was important in determining if wildfire ash and debris impacts were removed. Understanding how leachable arsenic may be in wildfire ash and debris was also important for determining if arsenic observed in some deeper soils could be attributed to the ash and debris. If elevated levels of arsenic were naturally occurring, it was important to determine the bioavailability as people reoccupy properties. Bioavailability and leachability of arsenic in wildfire ash and debris, the underlying soils, and nearby background soils unimpacted by wildfire ash and debris were assessed. Arsenic in wildfire ash was found to be much more bioavailable than naturally occurring arsenic. Leaching of arsenic from wildfire ash and debris was not observed. The information learned during the 2020 Oregon wildfire cleanup can benefit future wildfire cleanup efforts.

**5.17.T-06 Oklahoma City Playground Soil Contamination: An Environmental Justice Issue?**

***Sarah Hileman*** *and Jason B. Belden, Oklahoma State University*

The Greater Oklahoma City Urban Area is a melting pot of diversity both in socioeconomics and racial backgrounds. As expected in an urban setting, there are also elevated anthropogenic activities leading to environmental contamination. Polycyclic aromatic hydrocarbons (PAHs) and metals are generally ubiquitous in the environment and are often at higher levels in urban areas. Both persistent pollutants may correspond with atmospheric deposition, and therefore in urban soils these contaminants tend to be found in elevated levels due to proximity to sources such as vehicular traffic. Sampling in the Greater Oklahoma City Urban Area has indicated that PAH accumulation (and especially carcinogenic PAHs or caphs concentration) has been significant, and in many cases cPAH load has been measured above the USEPA's residential soil screening level of 110ppb. This occurrence has been noted across the city, as well as within public elementary school playgrounds: 138 public elementary schools across 15 school districts were examined for levels of polycyclic aromatic hydrocarbons (PAHs) and metals. Schools in this metropolitan range tend to have a high degree of concern, and there is the potential for oral exposure to these contaminants in sensitive age groups by way of hand-to-mouth actions involving soil. Carcinogenic PAHs in the playground soil were found to be elevated often above the recommended USEPA soil screening levels, and metals were found to be above the naturally occurring soil metals levels for the central region of Oklahoma. While these results indicate possible issues for children attending schools with higher levels of cPAHs and metals in the soils, they also pose a problem associated with disparities among the schools and districts. These schools have varying socioeconomic levels and racial diversity that provide an additional layer to the soil screening results that could be indicative of an Environmental Justice issue.

**5.17.T-07 Discussion 1 of 2: Soil Contaminants**
***Michael Simini***[1] *and Bonnie Brooks*[2]*, (1) U.S. Army DEVCOM Chemical Biological Center, (2) Washington State Department of Ecology*
The first portion of this session explores three different approaches to examine soil health. Some food for thought and discussion based on these studies:

1.Direct testing of invertebrate toxicity using soil dilutions, reproductive toxicity linked to gene expression, and bioaccumulation are discussed. What are the advantages and disadvantages of each method and can they be used interactively in a risk assessment?

2.Can any of these methods be tailored to specific soil types or regions using site-specific soil organisms and if so suggest how they may be adapted for particular site?

**5.17.T-08 Discussion 2 of 2: Soil Contaminants**
***Michael Simini***[1] *and Bonnie Brooks*[2]*, (1) U.S. Army DEVCOM Chemical Biological Center, (2) Washington State Department of Ecology*
In this portion of the session, methods to assess environmental fate, bioavailability and biomagnification of contaminants are presented for both ecological and human receptors. How can such methodologies be incorporated into risk assessments in each of the three scenarios?

**5.17.P Soil Contaminants: Fate, Bioavailability, Environmental Toxicology in Ecological and Human Health Risk Assessment**

**5.17.P-Tu143 Does temperature affect soil bacteria community in the presence of Naproxen & Ciprofloxacin?**
***Ezinne Osuji*** *and Deborah Carr, Texas Tech University*
Wastewater derived from domestic use commonly contains diverse pharmaceutical and personal care products (PPCP) that persists and accumulates in the soil. The effect of these pharmaceuticals on soil microbial community processes at different temperatures are poorly understood. Understanding environmental factors such as temperature that aid in degradation of these pharmaceutical will go a long way to elucidate ways to

reduce PPCP contamination in the soil environment. Ciprofloxacin (Cip), an antibiotics and naproxen (Npx), a non-steroidal anti-inflammatory drug, are two common PPCP in wastewater effluent. In this study, we examined how soil microbial community reacted when exposed to either of these pharmaceutical when incubated at different temperatures.

Soil was spiked with ciprofloxacin or naproxen and incubated for 90 days at 10°C and 30°C. After the 90 days incubation period, we determined changes in microbial community abundance at the two temperatures over time using 16S rRNA analysis. We used Qiime to determine any significant changes in composition and diversity of the microbial. Using taxonomy predicted from 16S sequencing, we inferred ecologically relevant function for each treatment from PICRUSt.

Alpha diversity calculated using Shannon diversity showed there was a significant increase in diversity in the Cip treatment at different temperatures, but the Npx treatment did not show a significant difference. The beta diversity measure, weighted Unifrac showed there was similarities between Npx treatment at 10°C & 30°C whereas there were dissimilarities between Npx at both temperatures, Cip at 10°C, and Cip at 30°C. The PCOA biplot showed temporal changes in the Cip treatment as well as dissimilarities amongst treatment type. Functional analysis showed that in all treatments, most function was for metabolism which includes xenobiotic degradation.

The change in soil bacteria community composition because of temperature change is dependent on the pharmaceutical involved. This shows that temperature influences the soil bacterial community at that time and may influence the degradation pattern of these chemicals in the soil. All life on earth including microorganisms experience the far-reaching effect of the climate change that includes higher mean temperatures which is likely to have detrimental effect on natural attenuation rates in which pollutants are transformed to less harmful forms through biodegradation, volatilization, dilution, sorption etc.

**5.17.P-Tu145 The Impact of Nano Zerovalent Iron on Remediating Mining Soils and Related Toxicity on Barley (Hordeum vulgare L.) and Tomato (Solanum lycopersicum L.)**

***Emily Christine Bowyer**, Luba Vasiluk and Beverley A. Hale, University of Guelph, Canada*

Metal pollution and accumulation in soils is increasing globally. Therefore, the need for in situ remediation technologies that are field applicable, effective and cost efficient is also increasing. The use of nano zerovalent iron (nZVI) as a remediation technology for soils is still an area of exploration. The current study compares the effectiveness of 5% nZVI at remediation of 27 soils with varying characteristics, including contamination with trace elements - especially As but also including Co, Cr, Cu, Ni, Pb and Zn. The study explores the impact of nZVI on total metal concentrations and $CaCl_2$ extractable concentrations. The expected outcome that nZVI would reduce the extractable metals was true in most but not all cases, increasing extractable metal in some. Germination indices (seedling emergence and root length) determined for barley (*Hordeum vulgare* L.) and tomato (*Solanum lycopersicum* L.) were improved for most but not all soils by addition of nZVI, suggesting that toxicity of nZVI itself is possible, depending on soil characteristics. These germination indices are being validated using 14-day studies (emergence, shoot length, root length and shoot/root dry weight) as growth response endpoints. The testable hypotheses of the study are that the metal concentration will decrease in the soils and plants treated with 5% nZVI but also that the germination and growth endpoints will decrease with 5% nZVI due to some toxicity.

**5.17.P-Tu147 Ecological Risk Assessment of the Levels of Polycyclic Aromatic Hydrocarbons in Soil Using Chronic Toxicity**

*Sooyeon Kim, Jong-Su Seo and **Hi Gyu Moon**, Korea Institute of Toxicology (KIT), Korea, Republic of (South)*

Polycyclic aromatic hydrocarbons (PAHs) are persistent organic pollutants having more than two benzene rings, and are known as representative carcinogenic environmental pollutants with high toxicity at low concentration.

In general, PAHs are primarily formed by anthropogenic activities including industrial pyrolysis, incomplete combustion of motor vehicle exhaust, fossil fuels, and wood. They are carbon-based nanomaterials with hydrophobicity and stable chemical structure, and the accumulation of PAHs onto the soil organic components is rapid. Moreover, the soil are important land-based input ways by rainfall and transitional zones where thier numerous pollutants flow into the river and sea. PAHs include representative 16 substances including naphthalene, phenanthrene (PHE), fluoranthene, and pyrene. Most of them are highly toxic, among them, PHE is classified as a substance that causes cancer in animals and humans. In addition, PHE is continuously flowing into the ocean by river water, it has low solubility and is not easily decomposed. Thus, it is absorbed well into the soil and can be accumulated at a high concentration. Therefore, risk assessment studies for PHE in soil that diffuse and remain in the environment from various sources are very important. This study aims to assess the ecological risk for PHE using chronic toxicity endpoints. A total of 8 chronic toxicity values tested with foreign species were based on the published literatures, which were simulated with the species sensitivity distribution (SSD) model for the derivation of chronic hazardous concentration for the most sensitive 5% of the species ($HC_5$). The results showed that the chronic $HC_5$ is 14.03 mg/kg, and the $PNEC_{chronic}$ divided by the appropriate assessment factor of 2 is 7.15 mg/kg. These results might be useful for the PAHs toxicity assessment in soil ecosystems.

**5.17.P-Tu148 Phytotoxic Effect Concentrations of Ce, Nd and Eu Added to Soil Relative to Total and Bioaccessible Soil REE Concentrations, and Tissue REE Accumulations**

*Amanda Pellegrino, Luba Vasiluk and **Beverley A. Hale**, University of Guelph, Canada*

Rare earth elements (REEs) are ‘technology critical’ thus are being increasingly extracted by mining, worldwide. As well, low concentrations (10-200 mg/kg) of some REEs in particular Ce, Nd, lanthanum (La), and samarium (Sm), are known to enhance crop production and can be added to soils intentionally. Low concentrations of REEs may also be inadvertently applied to crops with many P-fertilizers, because of geologic co-occurrence, specifically their tendency to form P-precipitates. At much higher concentrations (400-1500 mg/kg), REEs have been reported to reduce plant biomass. Toxicity thresholds for radish, tomato, and durum wheat responses (shoot length, shoot mass, root length) to Ce, Nd or Eu added to a high organic matter soil were determined from 14-day dose-response growth assays. $EC_{10}$ expressed as total soil [REE] had a more than ten-fold range, from 300 mg/kg to more than 8000 mg/kg. Averaged over all REEs and endpoints, $EC_{10}$ for wheat was higher than radish; tomato was of intermediate sensitivity and not different from either species. Averaged over all species and endpoints, the order from most to least toxic REE was Eu, Nd and Ce. When averaged over species and REE, the EC10 for the three endpoints were not different, between 2860 and 3400 mg/kg, suggesting that although roots typically accumulate more trace elements than shoots, plant health can be protected from REEs by assessing above-ground growth. Bioaccessibility of all three REEs in this soil were low (less than 0.10%), but Eu was five or six times more bioaccessible than Ce and Nd, which could account for its greater adverse effects on biota relative to total soil [REE]. Bioaccessibility-normalized $EC_{25}$ from other studies were very similar among all studies, demonstrating the importance of considering soil physico-chemical parameters as modifiers of soil REE toxicity. Tissue residue-effects assessment (TRA) $EC_{10}$ were the same for all REEs, suggesting that the ranking of Ce as the least toxic of these three REEs relates to inherently lower phytotoxicity, rather than lower accumulation by the plants. These three REEs are very similar in ionic radius and non-essential to plant physiology and their toxicity thresholds might be interchangeable.

## 5.18 The Other Bees: Approaches for the Pesticide Risk Assessment of Non-Apis Bees and Other Insect Pollinator Species

**5.18.T-01 Assessing the Exposure of Wild Pollinators to Pesticides Along an Agricultural Gradient Using Silicone Bands and Tissue Residues**

***Michelle L. Hladik**, Johanna Kraus and Kelly L. Smalling, U.S. Geological Survey*

Wild bees are important pollinators of agricultural crops, yet few studies have looked at the pesticide exposure in field-captured bees. Furthermore, although grasslands provide valuable habitat for wild bees, the broader implications of landscape-context (i.e., farming intensity) on bee exposure to pesticides within managed grasslands is limited. Conservation efforts have been implemented by the United States Department of Agriculture to create grasslands and pollinator-friendly habitat to support a diversity of pollinator species in altered (agricultural) landscapes. In 2019, we evaluated 24 conserved grassland fields located in Iowa, USA, with corn and soybeans as the dominant crops. Pesticides were measured in silicone bands (to capture aerial application/drift; placed on the landscape for one month prior to retrieval) and bee tissue (collected via sweep net). Each field was sampled twice, in July and August, to represent different parts of the growing season. Out of the 180 pesticides and degradates analyzed, 46 compounds were detected: 9 herbicides/degradates, 19 insecticides/degradates, 17 fungicides, and a plant growth regulator. Of the pesticides detected, 20 were observed in both silicone bands and bees, 16 in just the bands, and 10 in the bee tissue. As expected, herbicide detections were more frequent and concentrations were higher earlier in the growing season, while insecticides and fungicides were more frequently observed and at higher concentrations later in the season. Of the 10 pesticides detected only in the bees, five were degradates/metabolites, indicating the potential for the bees to transform the pesticides. Pesticide concentrations in the silicone bands increased with percent agriculture (within a 1000-meter radius) indicating a higher exposure potential in grasslands surrounded by a greater percentage of agriculture. In an agro-ecosystem, wild bees and other pollinators are being exposed to pesticides even in grasslands created to support and protect pollinator diversity.

**5.18.T-02 How can an automatic flight activity monitor contribute to our understanding of risk for pollinators?**

***Silvio Knaebe**[1], Volker Grimm[2], Katharina Schmidt[3] and Aline Mack[3], (1 )EAS Ecotox GmbH, Germany, (2) Helmholtz Centre for Environmental Research – UFZ, Germany, (3)Apic, Germany*

In recent decades, arthropod numbers have declined significantly. Pollinators are one of the most prominent groups that are clearly affected. Plant protection products are often named as one cause for this development, which is why they must be well tested before receiving market approval.

The risk to honey bees and other pollinators is determined in a standardised risk assessment. Methods of assessing impacts to honey bees are well established and includes snapshot checks of critical parameters in experiments.

Currently, there are rapid advances in technology to monitor the flight activity of a colony, i.e., the number of bees leaving and entering a hive. There is a great need for monitoring flight activity to gain knowledge about bee health, but also to estimate available food resources in the landscape. To this end, flight activity should preferably be monitored continuously, because that would allow the setting and measuring of threshold values for both lethal and sublethal effects. This type of monitoring could also include additional pollinators (i.e. bumble bees) either for testing or monitoring without the need for an observer to be present in the field. New Artificial Intelligence-based monitoring systems use machine learning to identify and count individual bees in real time based on of bees leaving and entering a hive. If this data was very precise, it would even allow to determine the number of bees which did not return and therefore quantify a decrease in colony strength. The AI-based monitoring systems are also able to track the number of pollen foragers entering the hive to assess foraging activity thought the amount of collected pollen and the share of pollen foragers among all bees entering the hive. In this way, exposure during an experiment can be estimated. In addition, pollen colour can be used to identify major crop species to further refine exposure estimates in the field, but also to assess landscapes for pollen diversity and supply abundance. Continuous assessment of pollen foraging would allow to determine if landscapes have sufficient pollen resources to support different pollinator species. Continuous monitoring of flight activity would also be useful to develop generalized models that can be corroborated with the data to determine if subtlethal effects could have a long-term impact on pollinators. One the monitoring system will be demonstrated and the design discussed. First results of applications will be presented.

**5.18.T-03 The fate of two radiolabeled compounds within a bumble bee microcolony**

***Daniel R Schmehl**, Ana R Cabrera and Randy Ripperger, Bayer CropScience LLC*

Pollinator toxicity and exposure are the key components for a quantitative pesticide risk assessment. There is a wealth of information that honey bees are protective of bumble bees in toxicity tests, yet pesticide exposure to bumble bee colonies is not well defined and data are limited. Our objective was to characterize the fate of two water-soluble radiolabeled compounds, C14-sucrose and C14-saccharin, when delivered via pollen and a sucrose solution to a bumble bee microcolony. We characterized C14-sucrose and C14-sacccharin exposure during the nest establishment phase (D0-10) and during part of the brood exposure phase in a microcolony brood test design (D10-31) to identify the primary matrices where these compounds are found. Most of the radiolabeled material was found in the nest, during both exposure scenarios for both sucrose solution and pollen dough. The amount of radiolabeled material found in the larvae, pupae, and adult workers was low. These results present an initial exposure characterization to bumble bees in a microcolony setting, and an indication that the bumble bee nest is a major structure where a compound may accumulate.

**5.18.T-04 Examining Soil as an Exposure Pathway in Nesting Female Solitary Bees**

***Christine Cairns Fortuin**, Elizabeth McCarty and Kamal J.K. Gandhi, University of Georgia*

Most wild bees in the United States utilize soil as a nesting substrate or as nesting material, however the risk pathway for agrochemical exposure from soil in the nest is poorly understood. *Osmia lignaria* Say is an economically important pollinator, whose interaction with soil and widespread distribution make them effective candidates for determining the role of contaminated soil on adult mortality and behavior, and larval developmental outcomes. Imidacloprid is a neonicotinoid pesticides which is commonly applied as a soil drench or chemigation application in nursery, orchard and forestry settings. We assessed impacts of acute contact with imidacloprid residue in soil at three concentrations based on residues which have been detected in soil at or near the application site in the first six months after a soil drench or chemigation: 50 ppb, 390 ppb and 780 ppb, and a control (0 ppb). We assessed acute contact with soil residue on reproductive output of adult female *O. lignaria*, and tested mortality after acute contact with two different soil moisture levels (20 % and 40% moisture) at each concentration of imidacloprid. In addition, we tested behavioral responses of adult females to treated soil utilizing choice experiments. In adult female *O. lignaria*, nesting activity was reduced by 42% for females exposed at 390 ppb and by 66% for females exposed at 780 ppb. Females treated at 780 ppb produced 40% fewer nest cells per day. Sex ratios of F1 generation were skewed toward male in the 50 ppb treatment group with 50% fewer females. In adult females, there were no mortality effects at 20% soil moisture for any level of imidacloprid, but at 40% soil moisture, mortality of females was >50% at all levels of imidacloprid. We found no evidence that *O. lignaria* females avoided any level of imidacloprid contamination when selecting soils for nesting, even at the highest residue level. These results suggest that a) acute exposure to imidacloprid residue in soil can have negative impacts on reproductive output of adult soil-interacting bees, b) mortality effects of acute contact by adult bees is relative to the degree of soil moisture, c) soil-interacting bees do not avoid interaction with or use of contaminated soils as nesting material, thus risk of exposure to both adult females and larvae are possible.

**5.18.T-05 Transfer of Pesticides from Solitary Bee Nesting Materials into Provisions: Laboratory Determination of Pesticide Rate Constants and Novel Distribution Ratios**

***Calvin Luu**[1], Kimberly J. Hageman[1], Theresa L. Pitts-Singer[2] and Diana Cox-Foster[2], (1) Utah State University, (2) U.S. Department of Agriculture*

The alfalfa leafcutting bee (*Megachile rotundata*) and blue orchard bee (*Osmia lignaria*) are commercially available solitary bees that have been considered in the development of pesticide risk assessments. One route of pesticide exposure for these cavity-nesting bees that is not considered in honeybee pesticide risk assessments is larval ingestion of pesticides sourced from contaminated nesting materials. The nesting material, which are alfalfa leaves for *M. rotundata* and soil for *O. lignaria*, can be contaminated from pesticide application. Larval provisions made by the mother bee are in direct contact with the nesting material. Therefore, nesting materials could leach pesticides into provisions; larvae could then ingest pesticides from contaminated provisions. However, the rate of transfer of pesticides from nesting material into provisions is unknown, nor is the risk of exposure. Therefore, the objective of this work is to determine the rate constant (*k*) of pesticide transfer from nesting material (alfalfa leaves and soil) into provisions and calculate the distribution ratio near equilibrium for pesticides between nesting materials and provisions ($D_{leaf\text{-}prov}$ and $D_{soil\text{-}prov}$). Laboratory studies were conducted by simulating the encompassing contact between *M. rotundata* provisions and alfalfa leaves, and the one surface contact of *O. lignaria* provisions and soils. Leaves were spiked with chlorpyrifos or λ-cyhalothrin for *M. rotundata* studies. Soils were spiked with λ-cyhalothrin, bifenthrin, or pyraclostrobin for *O. lignaria* studies. Pesticides were extracted from leaves, soils, and provisions using automated dispersive solid phase extraction combined with pressurized liquid extraction. Analysis of pesticides were completed using gas chromatography tandem mass spectrometry (GC-MS/MS). Current results reveal transfer of chlorpyrifos from leaves into provisions occurs; the rate constant for zeroth order provision uptake was $k = 0.0053$ µg/g $h^{-1}$ while the rate constant for second order dissipation from leaves was $k = 0.0027$ g/µg $h^{-1}$. The distribution ratio between leaf and provision ($D_{leaf\text{-}prov}$) after 7 days of contact was $\log(D_{leaf\text{-}prov}) = 1.022$. Ongoing work will yield results for pesticides and matrices stated above. Data collected from this work will be used to develop a model to estimate the concentration of pesticides in provisions, which will assist in estimating the risk of pesticide exposure on solitary bee larvae from contaminated nesting material.

**5.18.T-06 Use of caterpillars and adult butterflies for assessing toxicity and ecological risk of insecticides**

***Tham C. Hoang***[1] *and Gary M. Rand*[2]*, (1 )Auburn University, (2) Florida International University*

The worldwide use of pesticides (e.g., insecticides) to control insect populations raises concern of exposures, potential effects and risks to non-target insects and pollinators. Most research and testing for assessing the effects and risks of pesticides on non-target pollinators use the honeybee as the standard test species. Butterflies play a critical role in pollination of terrestrial ecosystems however, there is limited information on testing methods and risk assessment for this group. Our research with larval (5$^{th}$ instar caterpillar) and adult life stages of five Florida native butterfly species in the past 15 years showed that butterfly is a valuable non-target species for studying toxicity and assessing risks of insecticides to pollinators. Five butterfly species (common buckeye (*Junonia coenia*), white peacock (*Anartia jatrophae*), atala hairstreak (*Eumaeus atala*), painted lady (*Vanessa cardui*), zebra longwing (*Heliconius charitonius*)) were studied using different exposure routes (i.e., thorax, wing, ingestion) to various life stages (i.e., larval, adult) in the laboratory for toxicity testing and risk assessment. Our research demonstrated that both caterpillars and adult butterflies were more sensitive to insecticides than the honeybee. The sensitivity is dependent on exposure route, insecticide, and species of organism. In general, the 5$^{th}$ instar caterpillars were more sensitive to insecticides (naled, permethrin, dichlorvos) than adult butterflies. Permethrin was the most toxic, and naled was the least toxic to butterflies. Zebra longwing was the most sensitive to permethrin and naled, and white peacock was the most sensitive to dichlorvos. Regarding exposure route, painted lady, white peacock, and atala hairstreak were more sensitive to naled exposure via forewing than thorax exposure. However, thorax exposure to naled caused higher toxicity to common buckeye than forewing exposure. The toxicity produced by wing exposure emphasizes the importance of using butterflies for toxicity studies over the honeybee because the wing surface area of butterflies is typically much larger than the surface area of honeybees. For dietary exposure to caterpillars of common buckeye, white peacock, and atala hairstreak, naled was more toxic than permethrin and dichlorvos. Toxicity results were used for risk assessments using a probabilistic approach. Our risk assessment indicated that both caterpillars and adult butterflies are at risk of insecticide applications.

**5.18.T-07 Differential sensitivity and synergy in adult and larvae Lepidoptera**

***Claire Badder****, UK Centre for Ecology & Hydrology (UKCEH)*

Lepidoptera occupy an interesting position in pesticide management as they are pollinators in their adult life stage but crop pests as larvae. However, pest management strategies rarely consider differential sensitivity between these life stages. Differential sensitivity to pesticides may have many biological explanations. For example, the potential of a species to metabolise pesticides. Therefore, an awareness of the presence of metabolic enzymes such as cytochrome P450's (CYPS), glutathione-S-transferases (GSTs) and carboxylesterases in a species may hold an insight into the potential sensitivity or tolerances the species is likely to have towards chemical toxicants. Here, we aim to we determine the toxicity of the insecticides imidacloprid and cypermethrin and the fungicides, chlorothalonil and prochloraz to adult and larval stages of the cabbage moth (*Mamestra brassicae*).Using single chemical toxicity data and TKTD modelling, we aim to identify cases of synergism between cypermethrin and the fungicides in binary mixtures. Finally, we begin to explore any differences in sensitivity using the levels of expression of CYPS, GSTs and carboxylesterases in the transcriptome of the larvae and adult. Single chemical tests were analysed using the GUTS_RED_SD model and revealed fungicides had no effect on mortality on cabbage moth larvae or adults. However, the insecticide imidacloprid exerted a toxic effect on survival of adults but not larvae. Larval mixture testing identified synergy in larvae between cypermethrin and prochloraz, also cypermethrin and chlorothalonil. Adult mixture testing also identified synergism in cypermethrin and prochloraz but no interaction between cypermethrin and chlorothalonil. Mixture tests between imidacloprid and prochloraz are ongoing. Further, a catalogue was created of CYPS, GST's and carboxylesterases from the genome of cabbage moth larvae. Then, comparing the adult and larvae transcriptome we have identified several differences in expression of CYPs. Notably the presence of CYP4C3 which is expressed in 28 different forms in the cabbage moth transcriptome. We hypothesise that differences in sensitivity may begin to be explained by differences in expression of metabolic enzymes. We hope that this multi-faceted approach of lab exposure,GUTS modelling and transcriptomics provides an insight into the power of omics as a predictive tool of toxicity,particularly when used alongside traditional toxicity testing of pollinator species.

**5.18.T-08 SolBeePop: Assessing Risks of Pesticide Exposures to Populations of Solitary Bees in Agricultural Landscapes, a Modeling Approach**

***Amelie Schmolke***[1], *Nika Galic*[2], *Vanessa Roeben*[3], *Thomas G. Preuss*[3], *Mark Miles*[3] *and Silvia Hinarejos*[4], *(1) Waterborne Environmental, Inc., (2) Syngenta Crop Protection, Switzerland, (3) Bayer CropScience LLC, (4) Sumitomo Chemical Agro Europe, France*

Solitary bees, including both wild and managed populations, are important pollinators of crops and wild flower communites. Solitary bees can potentially be exposed to pesticides via multiple routes of exposure which may differ between species and between solitary bees and the Western honey bee (*Apis mellifera*) which is currently used as surrogate for risk assessments across bee species. Species-specific traits may additionally interact with the potential for exposures and effects, including, for instance, phenology, reproductive rates and flower preferences. We are presenting a population model for solitary bees in agricultural landscapes, SolBeePop. The model was developed to simulate a variety of species by using species-specific ecological traits as model parameterizations. Model parameterizations for several species (*Osmia bicornis*, *O. cornifrons*, *O. cornuta*, *O. lignaria*, *Megachile rotundata*, *Nomia melanderi*, and *Eucera (Peponapis) pruinosa*) were compiled from the literature whereby data availability varied by species. The model can simulate the diverse life cycles of the species and can be used to explore the importance of uncertainties in data to the population dynamics. Exposures to a pesticide through multiple exposure routes can be considered, such as nectar, pollen, and nesting materials. Effects are implemented using a simplified toxicokinetic-toxicodynamic model, BeeGUTS, adapted specifically for adult bees while an exposure-response functions is applied to simulate effects to developing in-nest life stages. We calibrated and validated the model with control data from semi-field studies conducted with *O. bicornis*. We applied the model across the model species to assess the impacts of different trait combinations on population dynamics, exposures and population-level effects in relevant landscape scenarios. The model provides a valuable tool for higher-tier pesticide risk assessments across species of solitary bees in agricultural landscapes.

**5.18.V The Other Bees: Approaches for the Pesticide Risk Assessment of Non-Apis Bees and Other Insect Pollinator Species**

**5.18.V-01 BeeGUTS - a TKTD Model for the Interpretation and Extrapolation of Bee Survival Data**

***Jan Baas***[1], *Benoît Goussen, Mark Miles*[2], *Thomas Preuss*[2], *Verena Taenzler*[2], *Vanessa Roeben*[2] *and Ivo Roessink*[1], *(1) Wageningen University & Research, Netherlands, (2)Bayer CropScience LLC*

Effects of pesticides on adult honey bees are currently assessed with standardized OECD tests. Three different types of tests are currently used acute and chronic oral exposure and acute contact. The typical exposure duration is 48 hrs in the acute tests and 240 hrs in the chronic tests. The tests are evaluated individually and the lowest value of any of the tests is taken for further risk assessment.

In practice this approach implies that the perceived sensitivity of a bee to a pesticide depends on the type of test. In addition the test results expressed as a 48 hr $LD_{50}$ (or the dose that kills 50% of the exposed bees after 48 hrs of exposure) or a 240 hr $LC_{50}$ (the concentration of the pesticide in the food that kills 50% of the exposed bees after 240 hrs of exposure) are not suitable to be used for field relevant conditions where exposure concentrations are not constant over time. Therefore, a mechanistic integrative assessment was developed where all tests can be interpreted and evaluated within one consistent framework with one set of parameter values. The developed approach uses a Toxicokinetic Toxicodynamic (TKTD) modelling framework based on the Generalized Unified Threshold model for Survival (GUTS) that takes into account the specifics of the test and the physiology of the bee; the BeeGUTS model.

Raw data were made available for chronic, acute oral and acute contact tests for 17 individual pesticides for honey bees. These test results were used to calibrate and validate the BeeGUTs model. It showed that the model was able to integrate the results of the three different exposure routes (acute oral and contact, and chronic oral) and interpret the different tests with one set of parameter values. This gives one single measure for the intrinsic sensitivity a bee to a pesticide regardless of the test or the exposure profile. Different species of bees could be incorporated in the modelling framework and subsequently their sensitivity could be compared to that of the honey bee, this showed that honey bees are consistently among the most sensitive species of bees for pesticide poisoning.

This new integrative approach moving from single point estimates of toxicity and exposure to a holistic link between exposure and effect will allow for a higher confidence of (honey) bee toxicity assessment in the future and a much-improved extrapolation potential of effects for different species and/or for field realistic exposure conditions.

**5.20.P Poster Only: Environmental Risk Assessment**

**5.20.P-Mo156 Polystyrene Micro/nanoplastic Concentration, Exposure Duration, Size, and Surface Functionalization Effects on the Uptake and Viability of Hepatic Cells**

*Amrita Banerjee, Lloyd O. Billey, Amy M. McGarvey and* ***Weilin L Shelver****, U.S. Department of Agriculture*

Micro/nanoplastics (MP/NP) are emerging contaminants found in diverse matrices including food and drinking water. Despite the exponential increase in research on the effects of MP/NP toward different biota, the information of their impact on food safety are scant. The liver serves a major role in clearance of xenobiotics and would function as one of the first organs encountered by ingested MP/NP. Worldwide, polystyrene is one of the most abundantly produced plastic polymers. Therefore, effects of polystyrene MP/NP on liver HepG2 cells were studied based on the role of concentration, particle size, surface functionalization, and time of exposure. Cellular responses to particles of various sizes (50 – 5000 nm) and surface functionalization (aminated, carboxylated or non-functionalized) were determined at different concentrations (0.1 - 100 µg/mL) and exposure periods (1 - 24 h). Smaller particles were internalized by HepG2 cells to a greater extent than larger particles with aminated particles showing a higher uptake than either the carboxylated or nonfunctionalized (NF) particles. The percentage uptake was greater at 10 µg/mL than 100 µg/mL concentrations indicating that the uptake process was saturable. Confocal microscopy images of cells corroborated quantitative uptake results. Cellular toxicity paralleled cellular uptake, with higher toxicity observed with the 50 and 100 nm sized aminated particles. The aminated particles were more toxic than the carboxyl or NF particles corresponding to the uptake studies. Measurement of Caspase-3 concentrations at 4 h showed increases relative to no particle treatment and caspase-3 concentration were decreased at 24 h relative to 4 h, but the measurements demonstrated the toxicity of the smaller particles was not mediated by apoptosis. Larger particles (500 nm -5000 nm) induced a greater increase of interleukin-8 (IL-8) concentrations and the levels increased from 4 h to 24 h treatment. This study shows that polystyrene particles can be harmful to hepatocytes, but the extent and mechanism of toxicity were dependent on particle size, surface functionalization, concentration, and length of exposure.

**5.20.P-Mo157 Application of U.S. EPA's Web-based Interspecies Correlation Estimation (Web-ICE) under the Toxic Substances Control Act (TSCA)**

***Kara Koehrn**[1], Molly Shuman-Goodier[2], Sandy Raimondo[1], Emily Vebrosky Nolan[1], Christopher Green[1], Jim Bressette[1] and Karen Eisenreich[1], (1) U.S. Environmental Protection Agency, (2) Department of Fish and Wildlife (DFW), Washington State University*

Without minimum data requirements under TSCA, EPA/OPPT often conducts risk evaluations using limited measured toxicity data. A novel modeling approach, Web-based Interspecies Correlation Estimation (Web-ICE), predicts toxicity values for environmental species that may be absent from a dataset, potentially providing a more robust dataset to estimate toxicity thresholds. Web-ICE is an online tool that estimates acute toxicity values for aquatic and terrestrial species using surrogate species data and least squared regressions. ICE models are developed from extensive, standardized datasets of acute toxicity, such as $EC/LC_{50}$ for aquatic species (e.g., fish and invertebrates) and $LD_{50}$ for terrestrial species (e.g., birds and mammals). Each ICE model represents the relationship of inherent sensitivity between a surrogate species (represented by the x-axis) and a predicted taxon (species, genus, or family represented by the y-axis). Web-ICE models use surrogate species sensitivity as an input to estimate the sensitivity of all available taxa. EPA/OPPT compared methods for calculating hazard thresholds for aquatic species using acute toxicity data. Results from the conventional lowest endpoint method were compared to results using a probabilistic approach with Web-ICE predictions and without Web-ICE predications. The use of Web-ICE to derive hazard thresholds provides several advantages over the lowest endpoint selection by predicting toxicity values for additional species, allowing for a probabilistic analysis and data-derived method of estimating uncertainty. Previous methods relied on fixed assessment factors to account for variation in toxicity across species, which have been criticized as not being reflective of the data available and having the potential to underestimate uncertainty, especially when only small datasets are available. This work will highlight the application of Web-ICE on chemicals with varying amounts of empirical toxicity data. Results for several chemicals show comparable hazard thresholds between the two approaches and indicate that Web-ICE can be used to improve hazard estimations in future chemical risk evaluations under TSCA. The views expressed in this abstract are solely those of the authors and do not represent the policies of EPA. Mention of trade names or commercial products should not be interpreted as an endorsement by EPA.

**5.20.P-Mo158 Arsenic species distribution in three commercially canned seafood samples**

***Shubhra Bhattacharjee**[1], Sharon Wanjiru[1], Jeremy D. Bailoo[1], Michael Findlater[2] and Amrika Deonarine[1], (1) Texas Tech University, (2) University of California, Merced*

Exposure to arsenic (As) is associated with health problems such as cardiovascular disease and cancers. Arsenic toxicity depends on its chemical form, and so understanding As speciation in dietary sources, such as seafood, is important for estimating risk. Arsenic species found in seafoods include $iAs^{III}$, $iAs^{V}$, arsenobetaine (AsB), dimethylarsinic acid ($DMA^{v}$), methylarsonic acid ($MMA^{v}$) and arsenolipids (AsL), which are toxic to cells, except AsB. Limited studies are available on As species distribution in seafoods, particularly for AsL compounds. Here, we investigated total As concentrations and As species distribution in three seafoods commonly consumed in the US; seven tuna, three salmon, and one clam samples collected from local markets in Lubbock, TX. Among the samples analyzed, 6 tuna, 2 salmon, and 1 clam samples were wild-caught, while

the remainder were farm-raised. AsL were first extracted with hexane and methanol/dichloromethane, followed by reverse-phase high-performance liquid chromatography-inductively coupled plasma mass spectrometry (HPLC-ICPMS). The remaining As species were analyzed by extraction with nitric acid followed by anion-exchange HPLC-ICPMS. Total As concentrations were quantified using acid digestion followed by ICPMS. Total As concentrations ranged from 0.02-4.6 mg/kg. All samples contained AsB and $DMA^v$, along with 2 unidentified water-soluble species. AsB was the most prevalent species, with concentrations ranging from 0.19-3.03 mg/kg (average 1.36 mg/kg), while $DMA^v$ concentrations ranged from 0.027-0.11 mg/kg (average 0.06 mg/kg). Three tuna samples also contained $MMA^v$ (0.01-0.04 mg/kg; average 0.02 mg/kg). Tuna contained the most AsL (0.003-0.41 mg/kg; average 0.06 mg/kg), followed by salmon (0.009-0.11 mg/kg; average 0.02 mg/kg) and clam (0.006-0.02 mg/kg; average 0.005 mg/kg). Tuna samples contained 11 different AsLs while salmon and clam samples contained 7 and 3 AsLs, . Our results suggest that organic As species are more prevalent in seafood than iAs. In comparison to the recommended iAs limit for infant foods (0.1 mg/kg), AsL and $DMA^v$ concentrations were elevated in 57% of tuna and 33% of salmon samples. However, there is no regulatory limit for organic As in foods, and further study is needed to gain a full understanding of the risk posed by seafood and to develop a regulatory policy, if needed.

**5.20.P-Mo159 The Ecological Protective Concentration Level (PCL) Database - an Online Tool for Streamlining Ecological Risk Assessments in Texas, USA**

***Brian S. Yates**, Brad Heim and W.J. (Jim) Rogers, West Texas A&M University*

Ecological risk assessment (ERA) is an integrated part of contaminated site management and often drives cleanup decisions. Performance of an accurate, integrated and well-researched ecological risk assessment is paramount to site remediation and closure. However, this process is often time and resource consumptive, requiring long periods of literature review on contaminant toxicity, fate and transport, and exposure profiles of native species. There is a major need for a tool which facilitates the process of Tier 2 or 3by generating accurate and defensible protective concentration levels (PCLs).

Over the past 20 years, West Texas A&M University (WTAMU) and the Texas Commission on Environmental Quality (TCEQ) have developed the PCL database, and the TCEQ continues to support improvements and maintenance of the Database through a contract with WTAMU. This database calculates accurate, defensible PCLs for at least120 chemicals of concern (COCs) and 113 species of wildlife native to Texas. These chemicals and wildlife were chosen based on discussions within the Database work group, and are representative of commonly found contaminants at Texas Risk Reduction Program sites. Each COC contains its own toxicological profile which allows the user to review relevant information on the COC's fate, transport, bioaccumulation, and toxicity. Species were chosen based on food webs presented in TCEQ ERA guidance and are divided amongst the seven major habitats in Texas, with an additional two “minor” habitats also represented. Each species has a file containing its own exposure factors, which contains input data on the species' ingestion rates, home range, and other life history information.

PCLs were developed based on protection of growth, reproduction, and mortality of the indicator organisms, leading to potentially tens of thousands of PCL calculations. Recently, the WTAMU/TCEQ team has revisted the methodology  for predicting bioaccumulation of COCs using empirically-derived models (ORNL, etc.). Integration of recent bioaccumulation models for inorganic compounds, polycyclic aromatic hydrocarbons, halogenated organics, and other COC classes including perfluorinated compounds will facilitate the calculation of defensible PCLs for receptors located throughout the state's diverse habitats. The PCL database is a useful tool in facilitating rapid and accurate ecological risk assessments and ensuring more cost-effective and scientifically defensible cleanup decisions.

**5.20.P-Mo160 Development of a chronic OTNE Species Sensitivity Distribution**

***Kristin A. Connors**[1], Jared Bozich[2] and Aurelia Lapczynski[3], (1) Procter & Gamble, (2) International Flavors*

*and Fragrances, (3) Research Institute for Fragrance Materials*
Octahydro-tetramethyl-naphthalenyl-ethanone (OTNE) is high volume fragrance ingredient used in products disposed down-the-drain such as cleaning and personal care products. A chronic Species Sensitivity Distribution (SSD) was performed to describe the inter-species variation in sensitivity to OTNE to reduce the uncertainty of lab to field effect level extrapolations. Species were selected to adhere to SSD criterion for taxonomic diversity and to represent a surface water exposure scenario. A total of 11 chronic ecotoxicity studies were completed including 3 species of algae (including green, blue-green, and diatom species), 1 aquatic macrophyte, 5 invertebrates (including an amphipod and insect), and 2 fish. The OTNE chronic data were fitted to a log-logistical function from which the $HC_5$ ($SSD_{0.05}$) and 95% confidence intervals were calculated. The quality of the dataset and the sensitivity and stability of the SSD were validated using recommended criteria and conventional statistical procedures. These included "leave-one-out" and "add-one-in" statistical simulations using hypothetical data. These evaluations demonstrated that the chronic toxicity data were highly ordered, and strongly adhered to statistical assumptions. The product of this research contributes to a robust probabilistic assessment of the aquatic hazard of OTNE.

**5.20.P-Mo162 Assessing the biodegradation and environmental risk from antibiotics in surface waters**
*Jeffrey Brenchley, Alice DiFazio,* ***Joan G. Tell****, Kevin Luczak, Lisa Ziv and Camila Polanco, Merck & Co., Inc.*
Antibiotics serve a critical public health role in combatting various bacterial infections worldwide. Low levels of antibiotics in surface waters due to patient use, improper disposal or manufacturing, may contribute to the spread of antibiotic resistance, as well as impact ecological communities. Despite the number of antibiotics on the market, very few have complete environmental fate and effects data sets. Our company has conducted studies on select antibiotics to help inform environmental risk assessment. These data, presented herein, coupled with data of measured environmental concentrations from available literature and Predicted No-Effect Concentrations developed by the Antimicrobial Resistance (AMR) Industry Alliance form the building blocks needed to complete environmental risk assessments of antibiotics that address not only ecological risk but also the spread of antibiotic resistance from an environmental dimension.

**5.20.P-Mo163 Towards the development of fragrance specific ecological Threshold of Toxicological concern (ecoTTC)**
*Aurelia Lapczynski[1],* ***Kristin A. Connors****[2] and Jared Bozich[3], (1) Research Institute for Fragrance Materials, (2) Procter & Gamble, (3) International Flavors and Fragrances*
Threshold for Toxicological Concern, or TTC, is a well-established and regulatory accepted concept in human safety to rapidly screen and assess data poor compounds and mixtures. TTCs are used to establish a de minimis exposure concentration, below which negligible risk is expected. The use of TTCs has been accepted in regulatory safety frameworks for human safety endpoints and is leveraged by RIFM (Research Institute for Fragrance Materials) to screen fragrance materials in human health safety assessments (e.g., skin endpoints). Efforts are underway to develop an analogous ecological TTC (ecoTTC) approach using distributions of Predicted No Effect Concentration (PNEC) values. The objective of this project is to examine the applicability of the ecoTTC approach for fragrance specific materials. The EnviroTox database was specifically built to support the development and creation of ecoTTCs. This database contains over 4200+ chemicals and over 80,000 aquatic toxicity results. All information was collected from publicly available sources and has not been independently verified for quality. The RIFM proprietary environmental hazard database was explored to further augment the chemical domain of EnviroTox for chemical domains relevant for fragrance ingredients. A fragrance ingredient database was constructed using the robust, high quality ecotoxicity data from the proprietary RIFM database. Unlike the EnviroTox database, all experimental results from the RIFM database were manually reviewed and curated. Studies were assessed for accuracy, data quality, and validity of test methodology. After manual review, 421 acute invert, algae, and fish studies were identified and 48 chronic invert, and fish studies were identified as relevant for ecoTTC development. In effort to create tailored fragrance specific ecoTTC, chemical-class and/or mode of action-specific ecoTTCs will be explored. It is

anticipated that the fragrance-specific ecoTTC will update RIFM environmental risk assessment framework to minimize unnecessary animal testing without compromising safety.

**5.20.P-Mo164 Quantitative weight-of-evidence approach for bioaccumulation assessment of volatile methylsiloxanes in aquatic and terrestrial species using the Bioaccumulation Assessment Tool**

***Jaeshin Kim***[1]*, Sami Belkhiria*[2] *and Kathleen Plotzke*[1]*, (1) The Dow Chemical Company, (2) Dow Silicones Belgium srl*

Bioaccumulation of a chemical is predicted or assessed using different metrics such as bioconcentration factor (BCF), bioaccumulation factor (BAF), biomagnification factor (BMF), and trophic magnification factor (TMF). The metrics have different criteria for bioaccumulation according to the design of the endpoint. It is not uncommon to obtain conflicting results using the different metrics. To evaluate bioaccumulation of cyclic and linear volatile methylsiloxanes (VMS: D4, D5, D6, L2, L3, L4, and L5) in aquatic and terrestrial species, we have employed the Bioaccumulation Assessment Tool (BAT, ver2.02) that facilitates the evaluation of multiple kinds of bioaccumulation data in a quantitative weight-of-evidence (QWoE) approach. The model assesses bioaccumulation potential with consideration of the relevance and reliability of each available data point. As they are used as chemical intermediates and can be found as ingredients in consumer products, their environmental concentrations are in a wide range in different media including biota, surface water, air, and sediment. Thus, there have been multiple laboratory- and field-based data available such as BCF, BMF and TMFs. In addition, the BAT can evaluate bioaccumulation potential using QSAR predicted biotransformation rates and BCFs from the EAS-E Suite (QSARINS, IFS, and OPERA) and the EPA CompTox Chemicals Dashboard. These predictions filled the knowledge gaps in cases of limited measurements. According to the criterion of BCF, VMS can be bioaccumulative or non-bioaccumulative depending on the mass and $K_{OW}$ values. However, BMF data of VMS supports non-bioaccumulative behaviors in both aquatic and terrestrial species. The use of predicted biotransformation rates and BCFs was useful for assessing bioaccumulation potential of the VMS in the BAT. The overall evaluation will be presented with all the measured and predicted bioaccumulation metrics.

**5.20.P-Mo165 Evaluation of Microfurnace Pyrolysis-GC-MS Method Refinements for Reliable Quantification of Tire and Road Wear Particles (TRWP) in Environmental Matrices**

***Sharlee More***[1]*, Stephanie A Thornton*[1]*, Julie Miller*[2]*, Rachel Young*[3]*, Kathy Chan*[4]*, Timothy R. Barber*[5] *and Kenneth Unice*[1]*, (1) Cardno ChemRisk now Stantec, (2) Cardno ChemRisk, (3) Stantec Consulting Services Inc., (4) ChemRisk, (5) ERM*

Polymer-based materials provide essential functionality in a number of commercial products, building materials, textiles, plastics, and tire treads. Plastic or elastomer-containing particles potentially associated with the use of these products have been detected in water, soil, sediment, and air. Recently, frameworks for the management of these particles in the environment have been proposed by various groups and agencies. These frameworks recognize that identifying and quantifying polymer content in environmental matrices is challenging due to particle morphology and physicochemical property availability. Specific to functionality critical for transportation mobility, tire and road wear particles (TRWP) are produced by abrasion at the interface of the pavement and tread surface and contain tread rubber with road mineral encrustations. Analytical methods capable of quantifying TRWP are required to assess the prevalence and environmental fate of these particles. However, complex organic background constituents in sediment or other environmental samples can make it difficult to accurately determine TRWP concentrations using current pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) methodologies. Thus, this work identified and evaluated potential method refinements for microfurnace Py-GC-MS, including chromatography parameter modification, chemical pretreatment, and thermal desorption using microfurnace for cryogenically-milled tread (CMTT) samples in an artificial sediment matrix. The tire tread dimer markers used for quantification were 4-vinylcyclohexene (4-VCH), a marker for styrene-butadiene rubber (SBR) and butadiene rubber (BR), 4-phenylcyclohexene (4-PCH), a marker for SBR, and dipentene (DP), a marker for natural rubber (NR) or isoprene. The resultant modifications included sample pretreatment with KOH and optional thermal desorption, along with optimization of GC temperature and mass analyzer settings. The refined method improved peak resolution with minimized matrix interferences with accuracy and precision consistent with those typically observed in environmental sample analysis. The method detection limit for an artificial sediment matrix was approximately 100 mg tread/kg sediment for a 10 mg sediment sample. Subsequently, TRWP concentrations were determined in various environmental samples (e.g., sediment, sediment trap, and retained solids), which supported the utility of microfurnace Py-GC-MS for complex environmental sample analysis.

**5.20.P-Mo166 Biosolids chemical risk assessment**

***David Tobias**, U.S. Environmental Protection Agency*

The Office of Water (OW) at the EPA has responsibility for evaluating microbial and chemical risks resulting from the use and disposal of biosolids, i.e., treated sewage sludge from wastewater treatment plants. OW has developed a human and ecological framework to assess the risks resulting from chemical contaminants in biosolids that are land applied to farm fields or disposed of via landfilling. Environmental pathways evaluated include direct contact with soil, migration to groundwater and surface water, and volatilization to the air. These pathways result in human exposures via diet, household water, soil ingestion, and inhalation; and ecological exposures to aquatic, benthic, soil, and terrestrial organisms. All of these pathways and exposures are estimated by the BioSolids Tool (BST) that OW has developed to screen chemicals for risk and to scope which pathways and receptors may need a full risk evaluation. The BST is a desktop application that allows the user to enter exposure, fate, and hazard properties to calculate risks. OW aims to screen all of the chemicals that have been found in biosolids for risks and to scope potential chemicals that need additional monitoring data.

**5.21 SARS-CoV-2: Pivoting from Watersheds to Sewersheds in Response to a Global Pandemic**

**5.21.T-01 Responding to a pandemic: Development of SARS-CoV-2 wastewater surveillance to support public health action.**

***Mark R. Servos**[1], Hadi Dhiyebi[1], Meghan Fuzzen[1], Carly Barbara Anna Sing-Judge[1], Yash Badlani[1], Heather Ikert[1], Nivetha Srikanthan[1], Samina Hayat[1], Nathanael Harper[1], Eli Zeeb[1], Hoang Dang[1], Patrick Breadner[1], Sean McLay[1], Leslie Bragg[1] and John Giesy[2], (1) University of Waterloo, Canada, (2) University of Saskatchewan, Canada*

The COVID-19 pandemic has touched almost every corner of the globe and caused serious health impacts within our communities. Early in the pandemic it was recognized that wastewater surveillance of SARS-CoV-2 viral fragments presented a potential tool for widespread community surveillance. This approach has the advantage that it is independent of clinical testing method, criteria, or eligibility and integrates everyone within the sewershed, including asymptomatic cases. Our research group rapidly responded to the needs of the public health agencies by pivoting research from environmental toxicology to assessment of trends of SARS-CoV-2 in wastewater. We worked closely with the municipalities, Public Health Units, including holding weekly meetings to jointly interpret the data and established public dashboards to rapidly disseminate the information. Regular sampling of influent (3-6 d/week) conducted at several wastewater sites in Ontario since the summer of 2020, has shown wastewater surveillance to be a useful tool to track community spread and parallels closely the reported clinical cases and hospitalization rates. Furthermore, the onset of the Omicron variant and limitations in clinical testing capacity in Ontario left wastewater as the only reliable method to track infections during the 5th and 6th waves of the pandemic. Wastewater also emerged as an effective way to rapidly monitor the spread of variants of concern (VOCs) and this information supported traditional public health data and interpretation. This work has been used by local authorities to inform and support public health actions to protect our communities.

**5.21.T-02 Comparison of buffer concentration and direct capture method for purification of viral nucleic acid for epidemiological surveillance of SARS-CoV-2**

***Laura M. Langan***[1]*, Robert Sean Norman*[2]*, Heidi K. Bojes*[3] *and Bryan W. Brooks*[1]*, (1) Baylor University, (2) Arnold School of Public Health, (3) Texas Department of State Health Services*
Following the identification and spread of SARS-CoV-2, scientists worldwide and especially the international water community rapidly developed methods to quantify the viral genetic signal in untreated water. Such methods now act as complementary tools to clinical testing, or as a replacement to clinical testing whereby individual testing has become less common over time. Due to variation in viral loads in wastewater, it is prudent to constantly evaluate whether alternative methods may confer increased sensitivity or reproducibility.

Wastewater samples from five locations including 4 university dormitories and one wastewater treatment plant were collected and viral loads quantified using a water concentration method (Zymo) with RNA extraction via Monarch total RNA miniprep kit or a direct capture method using a combination of speed vac and RNA purified using a semi-automated method. Viral loads were quantified for both methods using multiplexed probe-based RT-qPCR assays detecting N1, N2, PMMoV with two different one-step RT mastermixes. Recovery was estimated for each method using a process control spiked in prior to water concentration. In combination with viral quantification of the aforementioned targets (~40 mL), variable volumes of initial wastewater were used to evaluate the sensitivity of the method, with a further internal amplification control used to characterize and evaluate PCR inhibition. Recovery did not vary between methods and viral particulates could consistently be quantified in as little as 1 mL of initial wastewater, with a trend towards decreasing viral load based on initial starting material visible. However, for 3 of the 5 sampled sites, the water concentration method generally resulted in higher viral loads with the N1 target, however this trend was reversed with the N2 target, whereby the direct capture method resulted in higher viral load. The data underscores the importance of multiple targets and variable sized sampling locations in examining and characterizing variation in methodologies.

**5.21.T-03 Concatenated Plasmid for Improved Quantification and Normalization in COVID-19 Wastewater Surveillance**

***Minqing Ivy Yang***[1]*, Jianxian Sun*[1]*, Hadi Dhiyebi*[2]*, Mark R. Servos*[2]*, Hui Peng*[1] *and Elizabeth Edwards*[1]*, (1) University of Toronto, Canada, (2) University of Waterloo, Canada*
The single-stranded RNA enveloped virus SARS-CoV-2 causing COVID-19 is contained in infected individual's feces and ends up in sewage. Therefore, sewage wastewater samples provide an integrated signal for the entire population of the treatment plant's catchment that is independent of individual clinical testing. Quantification of viral signal in wastewater samples is usually carried out in a one-step reverse transcription-quantitative polymerase chain reaction (qPCR). Two subregions (N1 and N2) within the nucleocapsid protein-coding gene are popular SARS-CoV-2 signature biomarkers following CDC guidelines. A third reaction is often included to estimate the fecal matter concentration in a sample, which then is used to normalize the N1 and N2 signals for cross-sample comparisons. Pepper mild mottle virus (PMMoV) is one common fecal matter indicator. While several commercial standardized materials are available for N1 and N2, labs tend to generate their own PMMoV standards. The accuracy and variations of the PMMoV standards in different labs can have significant impact when comparing the PMMoV-normalized N1 and N2 values. We designed a concatenated plasmid with the insert containing the PMMoV, N1 and N2 partial genes in 1:1:1 ratio in the pTwist+Kan+High vector. The plasmid insert also contained the partial genes of other common targets in COVID-19 wastewater surveillance, including 229E, MHV, MS2 and crAssphage, and a T7 promoter to produce RNA. The synthetic concatenated plasmid was transformed into *E. Coli* and plated on LB agar plates. Colonies were selected and scaled up in LB broth. The sequence of the insert in the extracted plasmids was confirmed using Sanger sequencing. The plasmids were linearized by enzymatic digestion. Bioanalyzer and Qubit were applied to examine the quality and quantity of the plasmids. Specific qPCR was conducted to evaluate the completeness of linearization. The concentrations of the designed targets in the insert were assessed in qPCR against commercial SARS-CoV-2 standards as well as via digital PCR. Both qPCR and digital PCR revealed no non-specific reactions, and all designed targets were present in equal concentrations as expected. The concatenated plasmid

was used in interlab validation studies and was shown to help harmonize analyses for improved cross-lab comparisons in COVID-19 wastewater surveillance.

**5.21.T-04 The Utility of qPCR Assays for the Monitoring of Variants of SARS-CoV-2 in Wastewater Extracts**

*Meghan Fuzzen[1], **Carly Barbara Anna Sing-Judge**[1], Nathanael Harper[1], Hadi Dhiyebi[1], Heather Ikert[1], Shelley W. Peterson[2], Chand S. Mangat[2], Tyson E. Graber[3], Robert Delatolla[4] and Mark R. Servos[1], (1) University of Waterloo, Canada, (2) Public Health Agency of Canada, (3) Children's Hospital of Eastern Ontario Research Institute, Canada, (4) University of Ottawa, Canada*

Wastewater based epidemiology (WBE) has been shown throughout the COVID-19 pandemic to be an important source of information to determine the extent of SARS-CoV-2 currently circulating within a population. In addition to monitoring the total viral load, WBE can trace the prevalence of different variants of SARS-CoV-2 using targeted qPCR assays. Our research group has continuously developed new assays to determine which VOCs are circulating within the regions we are monitoring. In collaboration with partners across Canada, we have successfully monitored the emergence of the Alpha, Delta, Omicron, BA.2, and BA.2.12.1 lineages of SARS-CoV-2 in near real time in wastewater samples. This data has proven extremely useful to these communities, as the data can be produced more quickly than genomic sequencing and can also be performed more frequently due to the relatively low cost and logistics. The data produced using these methods aligns with that of the clinical qPCR and sequencing data for several communities as well, demonstrating the reliability of these methods. The data has been reported directly to Public Health Units to support decision making in these communities throughout the pandemic.

**5.21.T-05 Developing an interpretation model for wastewater SARS-CoV-2 viral load - a case of three Canadian Prairies cities**

***Femi F. Oloye**[1], Mohsen Asadi[1], Yuwei Xie[1], Jenna Cantin[1], Kerry McPhedran[1], Paul Jones[1], Mark R. Servos[2], John Giesy[1] and Markus Brinkmann[1], (1) University of Saskatchewan, Canada, (3) University of Waterloo, Canada*

Interpretation of the publicly available wastewater data is essential to help the public and government officials make informed decisions. Currently, the usage of data obtained from wastewater surveillance (WWS) has been challenging, given that WWS results may or may not be directly related to clinical data. A Wastewater Viral Load Risk Index (WWVLRI) was developed based on SARS-CoV-2 WWS viral load and available clinical data expressed as 7-day moving averages of active cases/hospitalizations to characterize COVID-19 situations within the community serving sewer system. The weekly average was classified as low risk when the viral load was less than 10,000 gene copies/100 mL and severe risk when the load was ≥ 50,000 gene copies/100 mL. Other values were classified as medium or high if they fell between high and low, but the rate of change was greater than 2-fold up or down. WWVLRI performed well across the Alpha, Delta, and Omicron variant waves in Saskatoon and the Delta and Omicron waves in Prince Albert and North Battleford. If discrepancies arise, values used in the index could be fine-tuned to each city by adjusting the WWVLRI parameters. The viral load correlated with active cases compared to inpatients hospitalizations and the number of patients in ICU. The highest correlation was obtained during Omicron waves, and the most negligible correlation was with the ICU. The correlation with the clinical cases varies among cities. The use of WWVLRI follows WWS and will support the "Living with COVID-19: Community-Level Surveillance and Risk Assessment Framework" created by the Government of Saskatchewan.

**5.21.T-06 Targeted Wastewater-Based Surveillance for COVID-19 Outbreaks in the Long-term care facilities in Edmonton, Canada**

***Xiaoli Pang**[1], Bonita E. Lee[1], Tiejun Gao[1], Yuanyuan Qiu[1], Eleanor Risling[2], Lorie A. Little[2], Douglas Faulder[2], Melissa Johnson[2], Krista Howden[2], Janelle Wallace[2], Michael Parkins[3], Casey Hubert[3], Kevin Frankowski[3], Steve Hrudey[1] and Christopher Sikora[2], (1) University of Alberta, Canada, (2) Alberta Health*

*Services, Canada, (3) University of Calgary, Canada*
Early identification of COVID-19 is a critical step to prevent outbreaks, protect vulnerable seniors and save lives during the pandemic. Wastewater (WW) surveillance of SARS-CoV-2 RNA (WBS SARS-CoV-2) has been reported as a leading indicator to clinical cases, which supports site-specific WBS SARS-CoV-2 for long-term care facilities (LTCF) as early warning. In this study, samples were collected from manholes of 9 targeted LTCF located in the city of Edmonton at 2-3 times per week from Jan 2021 to Feb 2022, processed, tested using RT-qPCR assays targeting N1 and N2 of the nucleocapsid gene and reported within 24 hours to medical officials responsible for health-related issues at LTCF. Upon receipt of SARS-CoV-2 RNA monitoring results in a WW sample from each LTCF, timely collaborative actions between the facility and outbreak management team have included: checking the COVID-19 outbreak status, initiation of outbreak investigations if appropriate, performing prevalence screening of COVID-19 if necessary, using additional precautions as needed, and investigation of transmission pathway.

A total of 873 WW samples were tested and 178 (20%) were positive for SARS-CoV-2 RNA with quantification ranging from 80 - 4.2 x$10^5$ RNA copies/100 ml in different LCTF. During the study, 48 suspected COVID-19 outbreaks were investigated and 17 were confirmed as COVID-19. We demonstrated strong correlation between WBS SARS-CoV-2 and confirmed COVID-19 cases reported in LTCF outbreaks. WBS SARS-CoV-2 is sensitive (detecting one infection in the whole facility), comprehensive (finding asymptomatic / pre-symptomatic / symptomatic case), non-invasive and unobtrusive with no interruption to the facility as "silent monitoring", and testing only requires a single sample for the whole facility. We have also learned of some challenges such as discordant results with some clinical cases not detected in WW because of non-daily sampling and rotating staff, and incontinent product usage in LTCF. On the other hand, some sewage samples tested positive without clinical cases identified likely due to asymptomatic/presymptomatic COVID-19 cases and visitors not tested during the outbreak investigation.

This study provides supporting evidence that WBS SARS-CoV-2 is a practical and useful tool at an institutional level before insidious spread of the virus within the facility and may protect resident seniors from devastating results of COVID-19 outbreaks.

**5.21.T-07 To Be Prepared for the Future, We Must First Examine What Lies Beneath-Wastewater Surveillance as a Tool to Monitor Pathogens. Exploring Methods, Data Trends, and the Future of Wastewater Surveillance**
***Golam Islam**, Ontario Tech University, Canada*
To overcome hardships of monitoring the transmission of severe acute respiratory syndrome coronavirus (SARS-CoV-2) in mass populations, researchers were compelled to look below, into the sewage pipes and develop an alternative pathogen surveillance tool to detect the genetic presence of SARS-CoV-2 in wastewater. Ontario Tech University's wastewater surveillance lab has been monitoring the prevalence and transmission of the SARS-CoV-2 virus in the Region of Durham and Simcoe County, Ontario since September 2020. This program currently encompasses 13 sampling sites which include 11 wastewater treatment plants and 2 upstream sewage pumping stations covering 750,000 residents. Using a Polyethylene Glycol-NaCl primary viral concentration step, RNA extraction and RT-qPCR, the observed wastewater trends of SARS CoV-2 have shown close association with the number of clinically diagnosed active cases during the alpha, delta and omicron waves within these communities. Our research has identified that crucial aspects of wastewater detection methods such as pasteurization, sample storage conditions and viral concentration methods can all affect the RT-qPCR detection of SARS-CoV-2 in wastewater (Islam et al., 2022). In addition, we analyzed vital factors which impact the interpretation of SARS CoV-2 wastewater data such as the applicability of using a fecal biomarker pepper mild mottle virus (PMMoV) to normalize the SARS-CoV-2 gene concentrations. Our results have demonstrated that in closed sewage systems, with very little infiltration, normalization using PMMoV may not be a significant factor in the interpretation of wastewater data. Furthermore, we have also examined the

effects of social restriction guidelines and vaccinations on wastewater trends that successfully limited the transmission of COVID-19. We are expanding our wastewater surveillance methods by designing novel multiplex assays that can target seasonal flu and flu-like viruses such as enterovirus, influenza A, and norovirus G II simultaneously with SARS-CoV-2. We have successfully detected the presence of Norovirus G II in eight influent wastewater samples over two weeks during a suspected outbreak in Simcoe County, Ontario using singleplex and duplex RT-qPCR assays. The emergence of wastewater-based surveillance of pathogens can be of great significance to public health organizations as a non-invasive and cost-effective manner to mitigate the risk of highly transmissible pathogens within communities.

**5.21.T-08 Panel Discussion: Lessons Learned and the Path Forward**

***Mark R. Servos*[1]*, Femi F. Oloye*[2] *and Laura M. Langan*[3]*, (1) University of Waterloo, Canada, (2) University of Saskatchewan, Canada, (3), Baylor University*

The panel discussion will engage in a dialogue about how researchers with diverse backgrounds were able to respond rapidly to the needs of a global pandemic. We will review the challenges faced, especially at the start of the pandemic, and the way that wastewater surveillance data was developed and used to support public health action. In addition, we will explore the lessons learned from this experience and highlight the opportunities for impact going forward. We will encourage all participant to share their experience with application of Wastewater-based Epidemiology (WSE) during the pandemic.

**5.21.P SARS-CoV-2: Pivoting from Watersheds to Sewersheds in Response to a Global Pandemic**

**5.21.P-Th130 Correlation between normalized SARS-CoV-2 and unnormalized viral load from three Canadian cities with different population sizes**

***Femi F. Oloye*[1]*, Yuwei Xie*[1]*, Mohsen Asadi*[1]*, Jenna Cantin*[1]*, Kerry McPhedran*[1]*, Mark R. Servos*[2]*, Paul Jones*[1]*, Markus Brinkmann*[1] *and John Giesy*[1]*, (1) University of Saskatchewan, Canada, (3) University of Waterloo, Canada*

Wastewater surveillance (WS) was employed to monitor the distribution of SARS-CoV-2 RNA in raw influent wastewater because WS is now a complementary tool for knowing the community incidence level of the COVID19. Data obtained from wastewater are not directly influenced by government regulations or the behaviours of any individuals. However, the data can be influenced by the dilution or concentration effect caused by water inflow and the population of the discharging community. Samples were collected using 24 h composite auto-samplers three times a week to determine the viral load of SARS-CoV-2 and chemical tracers. Viral loads were determined by qPCR, and whole-genome sequencing was used to screen for variants of concern (VOC). A high-performance liquid-mass spectrometer was used to determine the chemical tracers such as creatinine and acesulfame. Acesulfame was more stable than creatinine and was used to normalize the viral load. The normalized data has a Spearman correlation greater than 0.9, showing a high correlation with unnormalized data for the three cities. The dominant VOCs in the three cities were the same but with different proportions of sub-lineages. Sub-lineages of Delta were AY.12, AY.25, AY.27 and AY.93 in 2021, while the primary sub-lineage of Omicron was BA.1 in January 2022, and BA.2 subsequently became a trace-level sub-variant then the predominant VOC. Times that each VOC was first detected varied among cities; however, Saskatoon, with the largest population, was always the first to present new VOCs. Viral loads varied among cities, but there was no direct correlation with population size, possibly because of differences in flow regimes. The population is one factor that affects trends in the onset and development of local outbreaks during the pandemic. This might be due to demography, or the larger populations had greater potential for inter-and intra-country migration. Hence, WS data from larger cities can typically be used to indicate what to expect in smaller communities.

**5.21.P-Th131 Passive Sampling for the Detection of SARS-CoV-2 RNA in a University Residence Wastewater System**

***Blake Haskell**, Hadi Dhiyebi and Mark R. Servos, University of Waterloo, Canada*
Wastewater-based epidemiology has emerged at the forefront of the COVID-19 pandemic response as an additional tool for decision makers to employ effective management strategies. The detection of SARS-CoV-2 RNA in wastewater systems is a critical component of pandemic preparedness as routine surveillance has the potential to act as an 'early warning system' for outbreaks and is entirely independent of clinical testing limitations. This study employed a simple passive sampling approach to detect and quantify SARS-CoV-2 RNA in a university residence wastewater system using medical gauze as a sampling medium. Surveillance was performed three times per week from August 2021 to April 2022 and monitored over 4,000 students in 10 different on-campus residence buildings. Two targets in the N-gene of SARS-CoV-2 were routinely monitored, as well as the fecal indicator Pepper Mild Mottle Virus (PMMoV) for data normalization. Using a tiered sampling approach, N-gene viral wastewater signals were isolated to the building level to identify where infected individuals may reside. In one case, we were able to detect a single infection in a population of over 1000 students, one day before the student became symptomatic, and four days before clinical confirmation via PCR test. While the results demonstrated that the high sensitivity of the method was successful in detecting the presence or absence of SARS-CoV-2 RNA in wastewater, there were limitations to quantification and long-term trend analysis when SARS-CoV-2 levels approached or fell below the limit of quantification. High SARS-CoV-2 RNA loads in wastewater, as best demonstrated in January 2022 during the peak of the Omicron Variant, were most reliable for tracking real differences in the viral signal through time. By engaging multiple stakeholders involved in the on-campus pandemic response, decision-makers were equipped with an additional layer of evidence to support the enforcement or easing of restrictions. The surveillance data was also used to relay the relative risk to students living in affected communities.

**5.21.P-Th132 Advancing the Understanding of Partitioning Behaviour of SARS-CoV-2 in Wastewater**
***Patrick R. Breadner**[1], Nivetha Srikanthan[1], Hadi Dhiyebi[1], Samina Hayat[1], Leslie Bragg[1], Yuwei Xie[2], John Giesy[2] and Mark R. Servos[1], (1) University of Waterloo, Canada, (2) University of Saskatchewan, Canada*
The field of wastewater-based epidemiology has experienced rapid expansion and development since the onset of the COVID-19 pandemic. It has become a vital resource for tracking the spread of COVID-19 across communities. However, the absence of standardized methods across labs and remaining methodological questions can impact the interpretation of data for public health efforts. In particular, characterizing how RNA fragments of SARS-CoV-2 partition in wastewater is a central part of understanding its fate and behaviour in the wastewater. Additionally, various labs analyze either the solid or liquid fraction, and this has implications for the interpretation of analytical results and trends. The partitioning of SARS-CoV-2 RNA was examined in a series of experiments that were conducted using centrifugation with varied spin time and centrifugal force, polyethylene glycol precipitation, and filtration of wastewater. Partitioning of the endogenous pepper mild mottled virus (PMMoV) was also examined as it is commonly used to normalize the SARS-CoV-2 signal for fecal load in trend analysis. Additionally, two coronavirus surrogates (human coronavirus 229E and murine hepatitis virus) were analyzed as internal matrix spikes. Although SARS-CoV-2 has an affinity for solids, it was demonstrated that the total RNA copies of SARS-CoV-2 per wastewater sample split evenly between the liquid and solid fractions after centrifugation (i.e., 12,000 x *g* for 2 h). A longer and faster spin resulted in a shift in partitioning for all viruses toward the solid fraction except for PMMoV which remained liquid-dominant. This observation supports that the surrogates are more reflective of SARS-CoV-2 than the endogenous reference (PMMoV). Surprisingly, ultrafiltration devices were inconsistent with RNA copy estimation across filtrate volumes and competing brands illustrating potential biases that can exist across liquid-based approaches. Developing a better understanding of the fate of SARS-CoV-2 in wastewater and creating a foundation of best practices is key to supporting the current pandemic response but also to prepare for future potential infectious diseases.

**5.21.P-Th133 Impacts of Rotary Drum Thickener (RDT) on the Accurate Quantification of SAR-CoV-2 in Wastewater via RT-qPCR.**

***Yash Badlani**, Heather Ikert, Hadi Dhiyebi, Nivetha Srikanthan, Samina Hayat, Carly Barbara Anna Sing-Judge, Alice Gere, Joud Abu Farah and Mark R. Servos, University of Waterloo, Canada*
The implementation of Wastewater Based Epidemiology (WBE) in the current pandemic has been a crucial tool in understanding the level of COVID-19 in the population. However, there are technical challenges with the accurate quantification of viral RNA fragments via reverse transcription quantitative polymerase chain reaction (RT-qPCR). One of the challenges is the presence of substances which inhibit RT-qPCR, lowering the measured amount of viral RNA from the actual abundance. Wastewater samples from a wastewater treatment plant (WWTP) in Ontario, Canada (Site G) often contained substances which inhibited quantification of SARS-CoV-2 via RT-qPCR. The samples from Site G were determined to contain 3% of a return stream from the Rotary Drum Thickener (RDT) used to separate solid and liquid waste. The objective of this study was to determine if and at what level RDT in wastewater samples causes inhibition of RT-qPCR. To test the effects of the presence or absence of RDT, samples were collected at Site G over time under different conditions: during normal operating procedures (containing RDT), when the RDT supply was turned off, and when the RDT supply was resumed. To test the concentration dependent effects of RDT on inhibition, increasing concentrations of RDT from 0% to 4.6% were added to wastewater samples. Samples without RDT demonstrated little to no inhibition, whereas samples with RDT showed increasing inhibition of the RT-qPCR. These data suggest that the presence of RDT inhibits RT-qPCR, which limits the ability to accurately quantify viral RNA. The potential impact of inhibition caused by a variety of chemicals including those associated with process return streams need to be carefully considered. Extraction techniques to remove inhibitors, such as RDT may need to be applied before quantification via RT-qPCR. Quantification using digital PCR (dPCR) may also be a possible way to reduce the impacts of inhibition. Understanding how substances in wastewater impact accurate quantification of viruses is crucial to implementing WBE to support public health units in mitigating the impacts of COVID-19 and other diseases.

**5.21.P-Th134 Using Wastewater-Based Epidemiology (WBE) to Track the Prevalence of SARS-CoV-2 in Municipality Sewersheds**

*Hadi Dhiyebi[1], **Heather Ikert**[1], Nivetha Srikanthan[1], Samina Hayat[1], Meghan Fuzzen[1], Carly Barbara Anna Sing-Judge[1], Yash Badlani[1], Eli Zeeb[1], Hoang Dang[1], Patrick R. Breadner[1], Leslie Bragg[1], John Giesy[2] and Mark R. Servos[1], (1) University of Waterloo, Canada, (2) University of Saskatchewan, Canada*
The COVID-19 pandemic had a profound impact on communities around the world. Wastewater-based epidemiology (WBE) has emerged as an effective tool to support public health decisions and actions. Wastewater surveillance has many advantages because it is independent of clinical testing and captures the whole community including asymptomatic individuals. Methods were developed to measure SARS-CoV-2 viral fragments in wastewater using PEG precipitation with centrifugation, RNA extraction and qPCR focused on the CDC N1 and N2 gene targets. In addition, the pepper mild mottle virus (PMMoV) was measured and applied to normalize the SARS-CoV-2 signal. Extensive QA/QC has been applied, including testing for qPCR inhibition. Collaborating closely with Public Health Units and municipalities, a surveillance program was established in several communities in southern Ontario with over eight routine sites. Sampling has occurred three to six days per week since the summer of 2020 and reported directly and rapidly to the public health teams and made available on several public dashboards. Comparisons with conventional clinical testing have demonstrated that the use of WBE was very effective at tracking trends in the spread of the virus within a community. When the Omicron wave hit Ontario in December 2021, it overwhelmed the clinical testing, and this was further impacted by changes and reductions in the clinical testing programs. Wastewater surveillance of SARS-CoV-2 emerged as an independent, well-documented approach to continue monitoring the spread within the community. WBE continues to be one of the most effective ways to monitor SARS-CoV-2 in communities and the lessons learned will be important for future monitoring of existing and emerging diseases.

**5.21.P-Th135 Floatation-Based Sample Processing Method for Raw Wastewater Testing Bypassing Concentration Steps**

***Casey Wegner***[1], *Douglas Sieglaff*[2], *Aline Bridi*[2], *Shauntina Battiste*[2], *Jon Roussey*[1], *Brittany Macintyre*[1], *Tiffany Snow*[1], *Carolina Livi*[1], *Tarun Anumol*[2] *and Stephan Baumann*[2], *(1) Akadeum Life Sciences, (2) Agilent Technologies, Inc.*
Wastewater-based epidemiology became a widely known tool only recently in part due to the publication of COVID-19 online trackers and the focus of the media on the pandemic. Yet the analysis of viromes in wastewater has been widely applied for several decades in addition to traditional chemical analysis approaches also deployed. However, even though real time polymerase chain reaction based molecular detection methods are now mainstream in large and small labs alike, sampling and nucleic acid extraction procedures are not as of yet optimized to enable routine analysis and interpretation of results.

Tracking infectious agents at the community level in addition to testing individual samples provides valuable and actionable information around prevalence and distribution of human viruses. Wastewater testing allows for tracking the aggregate viral load within an area which will address low testing rates and asymptomatic cases being sensitive to tens of cases per hundreds of thousands.

Here we employ a floatation-based nucleic acid extraction method that allows for simple direct collection and lysis of total wastewater sample without requirement for pasteurization or filtration of solid components prior to analysis. Ultrafiltration is time consuming and removes solid component often containing the human viruses of interest. This method does not require pasteurization as an inactivating lysis buffer is added directly to the sample as the first step of the process. We characterize nucleic acid capture efficacy across a range of concentrations and resulting profile of the fragment sizes.

A proprietary microbubble separation tube was also developed to further improve workflows for wastewater extractions. The separation tube led to improved time to results as well as improved RNA recovery and qPCR signal compared to competing extraction methodologies. An additional advantage discovered during testing was reduced sample input needs to match the sensitivity of currently adopted precipitation and ultrafiltration-based methods. Using functionalized microbubbles designed to bind nucleic acids enables convenient and fast extraction, concentration and purification of RNA and DNA compatible with downstream genomics analysis.

With this novel microbubble approach total wastewater processing is simplified thought direct lysis offering same sensitivity using lower volumes to isolate nucleic acids for genomics analysis.

**5.21.P-Th136 Regional Analysis of Opioid Consumption with Wastewater-Based Epidemiology**
***Tyler Dow***[1], *Denina Simmons*[2], *Hélène LeBlanc*[2], *Andrea Kirkwood*[2] *and Jean-Paul Desaulnier*[2], *(1) University of Ontario Institute of Technology, Canada, (2) Ontario Tech University, Canada*
With the rapid increase in opioid-related harm over the past two decades, the opioid crisis has been identified as a leading health concern in Canada and has been exacerbated by the ongoing COVID-19 pandemic. In the Durham Region specifically, there were 93 deaths in 2020, which increased to 118 in 2021. Consequently, obtaining relevant information related to opioid use is critical to developing effective harm reduction strategies. Current data analysis methods involve medical records, population surveys, and crime statistics; many of which are expensive, cumbersome, and subject to potential biases. The study of public health related information in wastewater, termed 'wastewater-based epidemiology' (WBE), can produce an abundance of valuable information, making it an essential and cost-effective strategy for monitoring public health. Municipal wastewater contains many versatile parent compounds and metabolites, excreted by humans following the consumption and metabolism of legal and illegal substances. As the metabolic pathways of numerous illicit compounds are known, paired with data on wastewater flow within a given population, the concentrations of selected drug biomarkers can be used to estimate collective drug consumption. The aim of this study will be to develop and validate a reliable method to detect and quantify a selection of opioid drugs and their metabolites in wastewater within the Durham region using LC-MS/MS. Once per week, 24-hour composite wastewater

samples will be collected from each of the 15 wastewater treatment plants (WWTPs) located across the Durham Region. All composite influent samples will be spiked with a mixture of deuterated compounds for use as internal standards for quantification. Following filtration, the concentration of opioids and their metabolites will be performed using solid-phase extraction (SPE). Samples will then be analyzed in duplicate using liquid chromatography high-resolution tandem mass spectrometry (LC-HR-MS/MS). Chromatographic separation will be attained using a reverse-phase C18 analytical column, with a mobile phase consisting of gradient methanol/water. This research will have a robust economic impact by helping to inform intervention and preventative measures to mitigate harm from opioid consumption in the area. The information obtained from the proposed study would be used by the municipality to improve local public health interventions, which could then be a model for other agencies across Canada.

**5.21.P-Th137 Defining variability in formulas used to calculate gene copies in SARS-CoV-2 wastewater testing to aid comparability and increase reproducibility**

***Mia Ryon***[1]*, Laura M Langan*[1]*, Christopher Brennan*[2]*, Fallon Bain*[1]*, Aubree Miller*[1]*, Christine Snow*[1]*, Sean Norman*[3]*, Heidi K. Bojes*[4] *and Bryan W. Brooks*[1]*, (1) Baylor University, (2) Texas A&M University, (3) Arnold School of Public Health, (4) Texas Department of State Health Services*

Following the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), wastewater-based epidemiology (WBE) received heightened attention as an unbiased method for quantification of community level prevalence, irrespective of individual testing. Due to its increased application in the fight to minimize associated deaths, there has been an increase in WBE infrastructure globally. Due to various supply chain issues, this area has included variable methods of water concentration, RNA extraction and polymerase chain reaction (PCR). Whereas many studies have demonstrated the comparability of processing methods (e.g., water concentration, RNA extraction), limited studies have investigated the influence of variability in computational methods. Standard curve-based RT-qPCR methods are well established, but the steps from this measure to gene copies in wastewater is less well established. This reality was inevitable with such a quickly evolving field, yet variations in calculations could limit the extent to which studies can be compared. To identify if variations in formulas impact trends, and potentially relationships with epidemiological parameters, variability in the literature was quantified in a scoping review of existing WBE articles spanning Feb 2019- 2022, which resulted in 1012 articles. Following manual filtering and quality control, 287 publications were maintained to extract data for creation of a dataset. Key parameters were collected such as COVID-19 gc/L calculations (if reported), initial sample volume, and exogenous controls. Based on the formulas pulled from the review manuscripts, a shiny app was developed to visualize the variability in data calculations within a given data set using data generated over the past 6 months. In the first phase, non-normalized data was employed but later we plan to add other calculations including normalization, percent recovery, and aid in visualizing COVID-19 infectivity patterns. By gaining a stronger foundation in how we calculate the data collected from WBE, we can use this information to improve our understanding of studying disease markers within wastewater. We intend for this critical review and app to serve as a tool and stepping stone to improve an understanding of factors influencing COVID-19 spread and predictability of wastewater data to support public health interventions, but further as a platform that can then be applied to study other diseases through WBE.

**5.21.P-Th138 Developing a Normalization Tool to Better Correlate SARS-CoV-2 Concentrations in Wastewater With Clinical Cases.**

***Elliott Omer Holland-Chartrand***[1]*, Laury-Ann Dumoulin*[1]*, Marc-Denis Rioux*[1]*, Richard St-Louis*[1]*, Marie-Josée Ouellet*[1] *and Dominic Frigon*[2]*, (1) University of Quebec, Rimouski, Canada, (2) McGill University, Canada*

Wastewater-based epidemiology (WBE) has been used to assist various public health authorities manage and contain the COVID-19 pandemic with varying success. This passive surveillance approach can cover a large population with fewer resources compared to clinical diagnostic testing. However, one of the main challenges is the ability to accurately estimate clinical case trends in populations based on SARS-CoV-2 concentrations in wastewater. Five municipalities in Québec, Rimouski, Rivière-du-loup, Trois-Pistoles, Saint-Alexandre-de-

Kamouraska and Matane, with a population range of 2,000 to 49,000, were sampled during the spring of 2021. Normalization markers used were the Pepper Mild Mottle Virus (PMMoV) and wastewater treatment plant (WWTP) flow rate. Data shows there is a need for a more efficient normalization marker for this region. As a result, our objective is to quantify different biomarkers that could better normalize SARS-CoV-2 concentrations and therefore build a better model to estimate clinical cases in the population. Real Time quantitative Polymerase Chain Reaction (RT-qPCR) and liquid chromatography-tandem mass spectrometry will be used to quantify the selected biomarkers present in the wastewater samples. SARS-CoV-2 concentrations will be normalized using each new biomarker and will be correlated to clinical cases from the sampling period. The main hypothesis suggests the chosen biomarkers for this study will have comparable or higher correlation coefficients than the previously-used biomarkers in the province of Québec, therefore will be able to better predict the trends and estimate the number of clinical cases in the population. This study will not only establish a baseline for adequate biomarkers used in WBE, but will further help public health authorities of the region in their management of the COVID-19 pandemic as well as any future pandemic.

**5.21.P-Th139 Assessing the effectiveness of campus residence wastewater surveillance to drive public health interventions under different COVID-19 mitigation strategies**

***Joseph H. Bisesi Jr.***, *Andrew L. Rainey, Katherine Buschang, Amber O'Connor, Deirdre Honoria Doyle Love, Alexis Wormington, Rebecca L. Messcher, Julia C. Loeb, Sarah Robinson, Hunter Ponder, Sarah Waldo, Roy Williams, Jerne Shapiro, Elizabeth McAlister, Michael Lauzardo, John A. Lednicky, Anthony T. Maurelli and Tara Sabo-Attwood, University of Florida*

Wastewater-based epidemiology (WBE) has been utilized for outbreak monitoring and response efforts in the university setting across the globe during the COVID-19 pandemic. However, few studies have examined the impact of university policies on the effectiveness of wastewater based surveillance to identify cases and mitigate transmission. The objective of this study was to retrospectively assess the relationships between SARS-CoV-2 wastewater measurements and COVID-19 cases in residential buildings of a large university campus across two academic semesters (August,2020-May,2021) under different COVID-19 mitigation policies. Clinical case surveillance data of student residents was obtained from the university COVID-19 response program. We collected and processed the building-level effluent wastewater from over 30 residence facilities on weekly basis for the detection and quantification of SARS-CoV-2 genomic RNA by qRT-PCR. The odds of obtaining a positive wastewater sample significantly increased with higher COVID-19 clinical case numbers in the Fall 2020 academic semester (OR=1.50, P-Value=0.02), with higher odds in the Spring of 2021 academic semester (OR=2.63, P-Value<0.0001). We observed linear associations of overall SARS-CoV-2 wastewater concentrations and COVID-19 clinical cases (Parameter Estimate=1.2, P-Value=0.006), however, linear associations were not significant in the spring semester, when testing was recommended (not required) following a positive wastewater sample. Additionally, the results from our study indicate that positive predictive values and correlations between wastewater positive samples and cases in the corresponding dormitories were lower than other similar studies on college campuses. These other studies likely had higher positive predictive values and correlations due to compulsory clinical testing for all facility residents follow a positive wastewater sample from their residence building. Therefore, we recommend efforts to facilitate full participation in clinical SARS-CoV-2 testing among all residents of a given facility following a wastewater positive sample to successfully leverage WBE to optimize the benefits from this community-level surveillance tool.

**5.21.P-Th140 Wastewater surveillance for SARS-CoV-2 in a small coastal community: Effects of tourism on viral presence and variant identification among low prevalence populations**

***Andrew L. Rainey***[1], *Julia C. Loeb*[1], *Sarah Robinson*[1], *John A. Lednicky*[1], *John McPherson*[2], *Sue Colson*[3], *Michael Allen*[1], *Eric Coker*[1], *Tara Sabo-Attwood*[1], *Anthony T. Maurelli*[1] *and Joseph H. Bisesi Jr.*[1], *(1) University of Florida, (2) Cedar Key Water and Sewer District, (3) Cedar Key Chamber of Commerce,*

Wastewater-based epidemiology has been used to measure SARS-CoV-2 prevalence in cities worldwide as an indicator of community health, however, few longitudinal studies have followed SARS-CoV-2 in wastewater in small communities from the start of the pandemic or evaluated the influence of tourism on viral loads. Therefore the objective of this study was to use measurements of SARS-CoV-2 in wastewater to monitor viral trends and variants in a small island community over a twelve-month period beginning May 1, 2020, before the community re-opened to tourists. Wastewater samples were collected weekly and analyzed to detect and quantify SARS-CoV- 2 genome copies. Sanger sequencing was used to determine genome sequences from total RNA extracted from wastewater samples positive for SARS-CoV-2. Visitor data was collected from the local Chamber of Commerce. We performed Poisson and linear regression to determine if visitors to the Cedar Key Chamber of Commerce were positively associated with SARS-CoV-2-positive wastewater samples and the concentration of SARS-CoV-2 RNA. Results indicated that weekly wastewater samples were negative for SARS-CoV-2 until mid-July when positive samples were recorded in four of five consecutive weeks. Additional positive results were recorded in November and December 2020, as well as January, March, and April 2021. Tourism data revealed that the SARS-CoV-2 RNA concentration in wastewater increased by 1.06 Log10 genomic copies/L per 100 tourists weekly. Sequencing from six positive wastewater samples yielded two complete sequences of SARS-CoV-2, two overlapping sequences, and two low yield sequences. They show arrival of a new variant SARS-CoV-2 in January 2021. Our results demonstrate the utility of wastewater surveillance for SARS-CoV-2 in a small community. Wastewater surveillance and viral genome sequencing suggest that population mobility likely plays an important role in the introduction and circulation of SARS-CoV-2 variants among communities experiencing high tourism and who have a small population size.

## 5.21.V SARS-CoV-2: Pivoting from Watersheds to Sewersheds in Response to a Global Pandemic

### 5.21.V-02 Lead or Lag: Relationship between Wastewater-Based Surveillance of SARS-CoV-2 RNA and COVID-19 New Cases and Hospitalization in 11 Sewagesheds from Wild-Type to Omicron

***Bonita E. Lee***[1]*, Rhonda L. Rosychuk*[1]*, Erik Ellehoj*[2]*, Tiejun Gao*[1]*, Qiaozhi Li*[1]*, Yuanyuan Qiu*[1]*, Graham Tipples*[1]*, Mathew Diggle*[1]*, Nathan Zelyas*[1]*, Rasha Maal-Bared*[3]*, Christopher Sikora*[4]*, David Strong*[4]*, Jason Scarlett*[4]*, Deena Hinshaw*[4]*, Michael Parkins*[5]*, Casey Hubert*[5]*, Kevin Frankowski*[5]*, Nicholas Ashbolt*[6]*, Steve Hrudey*[1] *and Xiaoli Pang*[1]*, (1) University of Alberta, Canada, (2) Ellehoj Redmond Consulting, Canada, (3) EPCOR Water Services Inc., Canada, (4) Alberta Health Services, Canada, (5) University of Calgary, Canada, (6) Southern Cross University, Australia*

Wastewater-based surveillance (WBS) of SARS-CoV-2 RNA has been adopted as a supplementary tool during the COVID-19 pandemic in many jurisdictions, based on these key concepts: viral shedding starts in presymptomatic infection and is highest during initial infection, and there is a strong correlation between SARS-CoV-2 WBS and COVID-19 incidence. Utilization of SARS-CoV-2 WBS data varies depending on different settings and populations under surveillance. Studies have reported a lead time of 2-10 days by WBS as compared to COVID-19 clinical case based on best-fit correlation analyses of early pandemic with wild-type virus. During May 10, 2020 to Mar 15, 2022, 14 to 23 months of SARS-CoV-2 WBS data were generated from 2-3/week testing of wastewater samples collected from 12 wastewater treatment plants serving populations from 13,451 to 1.1 million in the province of Alberta, Canada. Using the population in 11 sewersheds mapped using Local Geographic Area boundaries, the objectives were to determine lead versus lag time between WBS and two variables: new cases of COVID-19 and COVID-19 hospitalization. The lead-lag analyses were performed by comparing the timelines between successive peaks of waves identified for SARS-CoV-2 WBS and the 2 variables. Waves as defined by peaks and pits were identified using turning point detection analysis after each dataset was normalized and smoothed (lowess). Up to top 5 peaks for all 3 variables were identified using Kendall's information theory. The predominant variant-of-concern of the peaks were determined by clinical variant testing of cases. A total of 3,021 wastewater samples were tested with an overall positivity rate of 65% (n=1950), ranging from 35% to 81% (median=66%) for the 11 sewersheds that had a range of 4 to 6 waves identified. The total number of new COVID-19 cases ranged from 425 in the sewershed with the shortest period of WBS (14 months) to 138,335 in the sewershed with the second highest served population and 23 months of WBS. Three sewersheds consistently showed a lead by WBS when compared to new COVID-19 cases for all identified peaks that ranged from 3 to 17 days. Variable lead and lag patterns were found in 8 sewersheds between WBS and new COVID-19 cases and between WBS and COVID-19 hospitalization in all 11 sewersheds for various waves with different variants overtime highlighting the complexity of factors affecting SARS-CoV-2 WBS, COVID-19 clinical surveillance and healthcare utilization.

**5.22.P Late Breaking Science: Environmental Risk Assessment**

**5.22.P-Mo192 Disrupted expressions of cortisol signaling-related genes in response to combined exposures of Cu and high temperature in zebrafish (Danio rerio)**
***Kiyun Park*** *and Ihn-Sil Kwak, Chonnam National University, Korea, Republic of (South)*
Fishes are frequently exposed to multiple stressors in combination introduced by intensive human activities or from natural sources that lead to the discharge of heavy metals into the aquatic environment. Cortisol signaling regulates a broad range of metabolic and physiological process. In the study, we investigated the potential effects of Cu exposures along with increased temperature in cortisol signaling pathway during zebrafish embryogenesis. Decreased survival rates were observed following combined exposure to high temperature and Cu. The cortisol levels in 96 hpf larvae following exposure to 0.003, 0.007, and 0.01 mg $L^{-1}$ Cu are measured at either 26 °C or 34 °C. The cortisol level is significantly increased by combined exposure to Cu and heat stress compared to only Cu exposure. In addition, the expression profiles of the fourteen cortisol and stress signaling related genes are altered by combined exposures of Cu and heat stress. Combination of heat and Cu exposures significantly increased transcriptional expressions of *Fdx1b*, *GR*, *Cyp2k22*, *GST*, *Crhb*, *SOD*, *Foxi3a,* and *Cyp11c1*, whereas mRNA expressions of *Hsd20b2*, *Hsd11b2, Cyp11a1, Cyp11b1, and Bcl-2* decreased by exposure to Cu and elevated temperature. These results suggest that realistic exposure to combined stressors induces hormonal disturbances through transcriptional alterations leading to cortisol signaling in zebrafish.

**5.22.P-Mo193 Responses of apoptotic p53 gene in 2,20,4,40-tetrabromodiphenyl ether (BDE-47)-induced oxidative stress in intertidal mud crab Macrophthalmus japonicus**
***Ihn-Sil Kwak*** *and Kiyun Park, Chonnam National University, Korea, Republic of (South)*
Persistent organic pollutants (POPs) were frequently detected in marine environments because of their wide application as brominated flame retardants in polyurethane plastics, electrical appliances, and expanded or extruded polystyrene foam. POPs such as 2,20,4,40-tetrabromodiphenyl ether (BDE-47), the most prevalent congener, is highly concentrated in the marine environment because of its water solubility and volatility and induces developmental toxicity in benthic organisms. In the present study, we evaluated *p53*-related apoptotic responses to BDE-47 in the intertidal mud crab *Macrophthalmus japonicus*. To do so, we characterized *M. japonicus p53* and evaluated basal levels of *p53* expression in different tissues. *M. japonicus p53* has conserved amino acid residues involving sites for protein dimerization and DNA and zinc binding. In phylogenetic analysis, the homology of the deduced *p53* amino acid sequence was not high (67–70%) among crabs, although *M. japonicus p53* formed a cluster with one clade with *p53* homologs from other crabs. Tissue distribution patterns revealed that the highest expression of *p53* mRNA transcripts was in the hepatopancreas of *M. japonicus* crabs. Exposure to BDE-47 induced antioxidant defenses to modulate oxidative stress through the upregulation of catalase expression. Furthermore, *p53* expression was generally upregulated in the hepatopancreas and gills of *M. japonicus* after exposure to most concentrations of BDE-47 for all exposure periods. The results in this study highlighs that exposure to BDE-47 may trigger the induction of cellular defense processes against oxidative stress, including DNA repair and apoptosis through the transcriptional upregulation of *p53* expression in *M. japonicus*.

**5.22.P-Mo194 Insects as a key to a more circular and sustainable planet – ENTOSAFE project**
***Diogo Filipe Nunes Cardoso***[1], *Rita Silva*[1], *José Nereu Pinto*[1], *Amid Mostafaie*[1], *Andreia Pereira*[1], *Patricia Silva*[1], *Amadeu Soares*[1], *Bryan W. Brooks*[2] *and Susana Loureiro*[1], *(1) University of Aveiro, Portugal, (2) Baylor University*

Farming insects have been presented as one of the best innovative sustainable solutions and a planet-friendly diet, but also challenging the re-use of sub-products such as bio-feedstocks and general wastes, reintroducing these components into the food value chain. Insects have a high content of nutrients and protein, and their use as food has great environmental advantages over conventional animal-source foods by introducing new sources of nutrients with lower environmental impact. This novel concept for generating feed and food is under zero waste policy and circular economy principles when coupled with sustainable waste management and agricultural practices. Nutritional biowaste (*e.g.*, non-commercialized vegetables and their residues) is used to feed insect larvae, serving as a food source. At the same time, organic waste is generated by the metabolic activity of the larvae/insects in the rearing facility, consisting of valuable biomass with high potential for agriculture as organic fertilizer. Aware of this, the "ENTOSAFE - Edible insects: From a sustainable food production to a food safety concern" FCT (Portuguese national funding agency for science, research and technology) project is being conducted, looking at the safety aspects of rearing insects by providing knowledge on the accumulation and transfer of potential contaminants in insects farmed in rearing facilities; this will directly contribute with data sets to the new legislation and regulatory limits regarding the presence of these compounds in the substrate. At the same time, the sustainability concerns of rearing insects are also covered by evaluating the effects of organic fertilizer on soil functions, namely water and nutrient retention, nutrient cycling, and plant performance in the amended soil. This presentation will then highlight the aspects and benefits of using insects with a key role in the re-use of waste and its conversion into valuable by-products, presenting the ENTOSAFE project's first results related to the safety and sustainability of this novel protein source.

**5.22.P-Mo195 Comparative Evaluation of Filtration and Imaging Properties of Filter Membranes for Microplastic Capture and Analysis**

***Jared Carter***[1]*, Teagan Zingg Horan*[1]*, Corinne Amato*[1]*, Erin Butler*[1]*, Gregory Madejski*[2] *and James Roussie*[1]*,*
*(1) SiMPore Inc., (2) Parverio Inc.*

Pollution by microplastics (MPs) in drinking, fresh and ocean waters, as well as food and beverages, is a growing problem that is now well-recognized in both popular media and scientific literature. Concerning levels of MPs have been found in food and local waters, as well as in human tissues. Due to a paucity of data, and lack of consensus regarding immediate and long-term toxicological effects on human health, MPs are an area of concern requiring further study. Consequently, there is a need for greater understanding of the performance characteristics of common MP analytical methods and where possible, for standardizing methods and reporting practices. Here, we report our work towards the comparative evaluation of filtration and imaging properties of five analytical membranes suitable for MP capture and analysis. This comparison was undertaken as part of an inter-laboratory methods evaluation study coordinated by the Southern California Coastal Water Research Project. We compared track-etched Polycarbonate with (PCTG) and without gold coating (PCTE), Polytetrafluoroethylene (PTFE), Porous Silicon (PS), and gold-coated MicroSlit Silicon Nitride membranes (MSSN-Au). Four of the filter types were purchased with a nominal 1.0 µm cut-off, except for PCTEG which was purchased with a 0.8 nominal cut-off. We examined the pore size characteristic and overall membrane structure of each filter type by electron microscopy to determine pore geometry and porosity characteristics. We compared clean water filtration rates and timed volume passage for each filter in comparison to its porosity and working surface area. We further compared optical microscopy imaging properties for each filter with model MP samples in both bright-field and fluorescent modes with accompanying Nile Red staining. In terms of absolute and surface area-normalized flow rates, our measurements ranked the filters in order of MSSN-Au > PTFE > PCTE > PCTEG > PS. Similarly, we found MSSN-Au filters compared favorably in optical microscopy modalities we examined. We further demonstrated the utility of the MSSN-AU filter type for use in surveying for MP contamination within the water production and distribution network in Monroe County, NY. These data demonstrate MSSN-AU membrane utility when incorporated in a standardized workflow for microplastics capture from clean water samples and on-membrane analysis.

**5.22.P-Mo196 Development of a Novel 20 µm Cut-off Microporous Silicon Nitride Membrane for Separating and Analyzing Microplastic Particles in Potable Water**

***Teagan Zingg Horan**, Jared Carter and James Roussie, SiMPore Inc.*

Track-etched polycarbonate (PCTE) membranes have been used as a filtration standard for Microplastic (MP) particle capture and analysis across a myriad of related studies. For instance, California's "Standard Operating Procedures for Extraction and Measurement by Raman Spectroscopy of Microplastic Particles in Drinking Water" recommends the use of 20 µm cut-off PCTE membranes for testing municipal water for MP levels. However, PCTE membranes suffer from inconsistent pore geometry and handling difficulty. The track-etch method creates pores of varying angles that complicate on-membrane particle analysis and sometimes create merged multi-pores that allow passage of MPs > 20 µm, while the membrane's overall ~3 µm thickness makes them prone to folding and wrinkling. To address these issues, a novel 20 µm gold-coated microporous silicon nitride (MPSN-Au) membrane was developed and compared directly against gold-coated PCTE membranes. We compared the physical properties of both PCTE-Au and MPSN-Au by electron microscopy, gas and water flux, as well as optical imaging and Raman spectroscopy studies of membrane-captured polystyrene (PS) particles. We found that on both an absolute and area-normalized basis, MPSN-Au membranes offered greater gas and water flux over PCTE-Au. The regular pore geometry of MPSN-Au membranes made particle imaging and spectral analysis more consistent and easier to discriminate between captured PS particles, when compared to PCTE-Au membranes. Importantly, the total time for a representative particle filtration and imaging workflow for both membranes from start to finish was compared. This testing determined that the total processing time (including filtration, automated image acquisition, and particle counting) was 161.56% faster on average for MPSN-Au than PCTE-Au membranes. Accounting for microscopy alone, in an 8-hour workday, 175 MPSN-Au membranes could be theoretically imaged, while only 31 PCTE-Au membranes could be imaged during the same time. Overall, these data demonstrate the utility of MPSN-Au membranes and suggest they can significantly improve testing time-related efficiency in all aspects of normal use-case situations as compared to PCTE-Au membranes.

**5.22.P-Mo197 Mercury Risk Evaluation, Risk Management and Risk Reduction Measures in the Arctic (ARCRISK); A Case Study of the Pasvik Watercourse, Northern Norway**

***Maeve McGovern**[1], Hans Fredrik Veiteberg Braaten[1], Cathrine Gundersen[1], Una Jermilova[2] and Jannicke Moe[1], (1) Norwegian Institute for Water Research (NIVA), Norway, (2) Trent University, Canada*

Mercury (Hg) contamination in Subarctic and Arctic regions is a significant environmental and human-health issue. The ARCRisk (Risk evaluation, risk reduction and risk management action plans for mercury in the Arctic – a circumpolar management approach) aims to develop a decision support tool together with stakeholders to mitigate Hg pollution. This tool will allow prediction of how ecosystems might respond to both reductions in anthropogenic mercury emissions and releases brought about by new regulations or changes to the global mercury cycle as a result of climate change.

Here, we present results from the Norwegian case-study system, the Pasvik watercourse, where 30 years of monitoring and research data were synthesized for incorporation in a Bayesian-Network Relative Risk model (BN-RRM) for identification of risk reduction measures for Hg releases into the Pasvik watercourse.

Located to the far northeast of Norway along the border between Norway, Russia, and Finland, the Pasvik watercourse receives contamination both through local sources, and long-range atmospheric transport, with elevated levels of Hg documented in water, sediments, and fish. The major local source of mercury is the Pechenganikel Mining and Metallurgical Combine in the city of Nikel, which likely releases contaminants directly into Kuetsjarvi Lake which is part of the Pasvik watercourse.

Hg concentrations in water, sediments and fish upstream and downstream of the city of Nickel are compared spatially and temporally. Preliminary analyses suggest that fish weight, Hg deposition and aqueous carbon are important for predicting Hg concentrations in fish from year 2000 to 2020. Temporal trend analysis showed slightly increasing fish Hg concentrations in this same time period. Next steps include the development of a Bayesian-Network Relative Risk model (BN-RRM) to identify targeted risk reduction measures for Hg releases, from sources including catchment soils, and local pollution, to the Pasvik watercourse. The final model will be used to test various future climate change and emission scenarios, and aid local management in protecting water resources used for drinking water, and for recreational and commercial fishing.

**5.22.P-Mo198 Drosophila Exposed to Polystyrene Microplastics and Nanoplastics Reveals Potential Implications for Human Health**

***Rachel Maria Sorensen****, Alicia Taylor, Alyssa M. Hohman, Kevin Cortes, Derrick K. Rollins, Eric Riddell, Elizabeth McNeill and Boris Jovanovic, Iowa State University*

Each day, the typical American may ingest up to 467,000 times the United States Food and Drug Administration's recommended daily dose of recyclable plastic in food in terms of all possible oral exposure of plastic. As microplastics and nanoplastics are able to interact at the cellular level in organisms, model species need to be used to elucidate possible toxicological effects in humans. Here, *Drosophila melanogaster* was exposed via diet to nanoplastic and microplastic and used as a model species to evaluate potential toxicological effects on development time, fecundity, net reproductive rate, generation times, emergence rate, lifespan, heart rate, and respiratory rate. In addition, for the first time, the number of microplastics ingested by a fly were quantified. There were no statistically significant differences in development time, net reproductive rate, generation times, emergence rate, and lifespan.  Results indicated that exposure to 50 nm nanoplastics decreased fecundity at 5, 14, and 20 days while both microplastics and nanoplastics differed from control for the last 20 days of assay. The total number of eggs in a life time showed statistically significant difference of less eggs present than the control at the end of the experiment. Sexually dimorphic changes to heart function and morphology in response to particle size were observed. Female *Drosophila* exposed to nanoplastics showed a decrease in their heart rate and systolic intervals. Male *Drosophila* exposed to microplastics had decreased systolic interval time and changes to heart size as measured by diastolic/systolic diameter. When evaluating the effect of metabolic rate and water loss for respiratory rate, results revealed that microplastic exposure decreased metabolic rates compared to controls. These results indicate that exposure to microplastics and nanoplastics cause effects in three major organ systems- respiratory, circulatory, and reproductive.

**5.22.P-Mo199 App Dates Tool: Automated Label-Compliant Application Date Assignment and Parameterization of Pesticide in Water Calculator (PWC) Batch Input Files**

***Logan Insinga**[1], Steve Kay[2] and Dean Desmarteau[3], (1) Applied Analysis Solutions LLC, (2) Pyxis Regulatory Consulting, Inc. / Generic Endangered Species Task Force (GESTF), (3) Waterborne Environmental, Inc., United States*

Endangered species risk assessments for pesticides use prospective models to simulate exposure and aquatic environmental fate. The USEPA's Pesticide in Water Calculator (PWC) simulates pesticide applications to land surfaces using the Pesticide Root Zone Model (PRZM) and the subsequent transport to and fate in surface water bodies using the Variable Volume Water Model (VVWM). Pesticides typically have diverse uses and restrictions that vary based on the use site, reapplication intervals, region, and time of year. Although PWC does accept a batch file to assist in automation, manual parameterization of numerous model runs is time-prohibitive and prone to human error. Specifically, defining application information (i.e., application dates and rates) is cumbersome due to the agronomic restrictions that are unique to use site and region. Agronomic restrictions include total pesticide amount and number of application limitations on an annual and interval specific (e.g., pre-emergence or post-emergence) basis, as well as multiple application rates with rate-specific limitations (i.e., number of applications and temporal windows), minimum reapplication intervals, and pre-harvest intervals. In addition, efforts to ensure modeling is conservative (e.g., simulation of applications during wettest months of the year and maximizing application rates) further complicate date assignment logic. The Generic Endangered Species Task Force (GESTF) have developed the App Dates Tool to automate batch file preparation for PWC modeling. Specifically, the App Dates Tool features a robust algorithm that generates label-compliant application dates and rates for a wide variety of use sites and regions. Landscape scale refinements can be implemented via the tool's graphical user interface where the user defines drift factors that correspond to various transport mechanisms (e.g., aerial, airblast, ground), distances, and receiving water body. The App Dates Tool to has been successfully used to parameterize a national set of PWC runs (n > 30,000) in a manner that was efficient, transparent, and repeatable.

**5.22.P-Mo200 Incorporating toxicokinetics into aquatic population models for chemical screening and prioritization**

***Tom T. Purucker**, U.S. Environmental Protection Agency*

High throughput toxicokinetic (HTTK) models are used for human health screening and prioritization of chemical exposures. However, direct adoption of similar workflows for other species are seen as lacking in ecological relevance. Translation of organismal-level hazard to population level impacts is the key missing component, yet toxicokinetic models are rarely embedded in population models at a scale necessary to inform chemical prioritization. This poster focuses on modeling linkages between toxicokinetic algorithms and aquatic population models to enable ecologically relevant chemical prioritization. Details are presented on conceptual model construction, algorithm selection, and parameterization for a relative risk evaluation of pesticides. The presentation is based on the Population Model Guidance, Use, Interpretation and Development (Pop-GUIDE) framework, an implementation of a systematic approach for model development and evaluation in a population modeling context. A common aquatic test species, the fathead minnow (*Pimephales promelas*), is chosen to represent a normalizing species for prioritization. The chemical domain is limited to classes of pesticides with readily available parameter information.

**5.22.P-Mo201 Potential human health risk of human pharmaceuticals accumulated in fish species of human consumption from Argentina**

***Macarena Gisele Rojo**[1,2] and Pedro Carriquiriborde[2], (1) Baylor University, (2) Centro de Investigaciones del Medioambiente (CIM)/CONICET-UNLP, Argentina*

Uruguay River and Río de la Plata Estuary are relevant aquatic systems within the Rio de La Plata Basin, one of the biggest in the world. Several cities and the Metropolitan Area of Buenos Aires discharge the raw wastewater into the basin. Commercial, recreational, and artisanal fishing are activities of population interest in the region. Human pharmaceuticals (HPs) have been detected in receiving wastewaters from the basin in the order of $\mu g\ L^{-1}$ and maximum concentrations in fish for human consumption in the range of 1 to 71.6 $\mu g\ Kg^{-1}$. This study aimed to assess the human health risk associated with the consumption of 10 detected HPs in fish tissue, collected in 3 sectors of the Rio de La Plata Basin: S1 along the Uruguay River, S2 in the lower Uruguay River, and S3 in the inner Río de La Plata. Species studied were *M. obtusidens*, *P. lineatus*, *P. maculatus*, and *S. brasiliensis*. MEC/PNEC ratio was used to obtain the "Risk Ratio" (RQ). MEC was the detected concentration of HPs in the fish muscle. PNEC was obtained from Acceptable Daily Intake (ADI) found in the literature for the compounds studied and combined with standards referring to potential fish consumption using human exposure parameters recommended by EPA guide, U.S. RQ was calculated as medium (RQ med) and maximum risk (RQ max) using average and maximum concentrations, respectively. Risk estimation for children and adults was calculated separately, being 1 as the limit value. RQ med ratios for children and adults were in the range of 2E-06 to 4E-03. ATE and SIL were exeptions, showing values in the range of 9E-06 to 4E-02 and 1E-03 to 1E-01, respectively. Overall RQ max values were between 2E-06 and 4E-03, but values of 2E-05 to 6E-02 and 4E-03 to 1E-01 were reported for ATE and SIL, respectively. Species collected in S2 and S3 showed the highest RQ max. Data such as local consumption rates in children and adults, portion size, effects of cooking, and effect of mixtures, were unavailable and should be relevant factors to be included in the future to obtain a more accurate assessment and prevent underestimation. HPs detected in this study are not established by national and international legislation. MEC for ATE, ENA, and SIL were above the Maximum Residue Limit (MRLs) of 5 $\mu g\ Kg^{-1}$, for diclofenac and carazolol established in bovine and pork muscle by EU No. 37/2010, SENASA

559/2011 and CODEX. Data generated in this study could contribute to future regulations on fish consumption in the region.

### 5.22.V Late Breaking Science: Environmental Risk Assessment

#### 5.22.V-01 The Implementation of a Predictive Spatiotemporal Model for Environmental Impact Assessment of Chemicals in Terrestrial and Aquatic Environments

***Parisa Falakdin**, Elisa Terzaghi and Antonio Di Guardo, University of Insubria, Italy*

Multimedia fate models are important tools for the simulation of the fate and transport of organic chemicals in multiple environmental media. The incorporation of environmental heterogeneity and dynamicity into these models is beneficial for better prediction of environmental levels and therefore chemical impact and risk assessment. A recently developed dynamic multiple box multimedia fate model (Gridded-SoilPlusVeg) was implemented to account for the environmental variation and the effect of directional advective transport of chemicals towards different compartments and geographical locations. As a result, the model provided the concentration values for the upper air, lower air, leaves, and superficial soil compartments for each grid cell. The model calculates the chemical fate processes among different environmental compartments such as deposition, volatilization, diffusion, degradation, and runoff. DynA model is a fugacity-based model developed to predict the fate of organic chemicals in a dynamic water-sediment system. Both models were used together to simulate a Northern Italy scenario where several water bodies exist (Lake Maggiore, Lake Lugano, and Lake Como). A chemical plant located in a small town (Pieve Vergonte in Ossola valley) produced and emitted DDTs for around 50 years. Later, Ossola valley was recognized as a contaminated site of national relevance (SIN Pieve Vergonte). Several sampling campaigns were conducted in the surrounding area in the years 2001 and 2011 and environmental samples of water, sediments, soil, and leaves were collected. The Gridded-SoilPlusVeg model was run for p,p'-DDT during and decades after the cease of production (a total of 100 years) for a study area of 200 km X 200 km. The contribution of this chemical plant to the water bodies was calculated by incorporating the deposition fluxes obtained from the Gridded-SoilPlusVeg model into the DynA model in order to calculate the concentrations in water and sediments. The results of both simulations were compared with the monitoring and literature data for water, sediments, soil, leaves, and air. This new spatial model accounts for atmospheric, geographical, and environmental variation within a multimedia fate model platform to include more realism in modeling and predicts various levels for a local and regional scale scenario.

#### 5.22.V-02 Refining Pesticide Exposure and Risk Assessments for Endangered Birds and Mammals: Working Within a Regulatory Framework

***John Marton**, Twyla Michelle Blickley and Patrick Havens, Corteva Agriscience*

In January 2022, the United States Environmental Protection (US EPA) released a policy that all new pesticide active ingredients would complete an Effects Determination for endangered and threatened (i.e., listed) species as a precondition to registration, thus satisfying the Endangered Species Act (1973). The process for evaluating listed bird and mammal species is similar to that of non-listed species undergoing a FIFRA assessment. These risk assessments rely on highly conservative exposure assumptions via US EPA's T-REX model, which offers little room for refinements based on a given species' biology and ecology. Further, these conservative assumptions are not always realistic or representative and can lead to restrictions at the county-level, thereby putting undue burdens on farmers while offering limited, if any, protection to the species. However, recent decisions by US EPA have offered insights into potential refinements that, while still conservative, allow for more realistic exposure and risk characterizations. For example, the dicamba decision (2020) introduced the feeding-depuration model for the American burying beetle (ABB) which models bird and mammal metabolism of a given chemical in contrast to T-REX which does not incorporate any metabolism. This model was utilized in the Enlist decision (2022) to refine exposure to the ABB following consumption of exposed bird or mammal carrion. Similarly, the Enlist decisions (2016, 2022) utilized more taxa-specific allometric equations and dietary assumptions (e.g., caloric needs based on feeding, caloric content and assimilation efficiency for specific dietary items, etc.) to estimate species-specific exposure and hence chemical burdens to listed birds and mammals. This presentation will explore case studies showing how these two approaches can be combined to more accurately represent exposure to listed birds and mammals, while still remaining within the regulatory framework set forth by the US EPA. Ultimately, this process will set the stage for new active registrations by advancing the best available scientific approaches for risk assessment refinement so that targeted, effective mitigations are enacted that protect the species while allowing farmers access to a greater range of plant protection solutions.

**5.22.V-03 Modeling Nontarget Aquatic Exposures to Wood Preservative Pesticides: Revising a Screening Level Approach**

***Dana K. Sackett****, Blossom Catacutan, Emily Saunders, Chuck Peck, Sophia Hu, James Breithaupt, Elizabeth Donovan, Jeanette Martinez and Melissa Panger, U.S. Environmental Protection Agency*

The United States Environmental Protection Agency's (EPA) Office of Pesticide Programs Antimicrobials Division is revising its modeling approach to assess screening-level risks to non-target aquatic organisms from pressure-treated wood preservatives. The current approach assumes that pressure-treated wood used to build docks represents the highest exposure potential to aquatic ecosystems, and that pesticide chemicals leach from a single treated 6m dock (6m x 1.2m x 0.05m) into a 1-hectare (ha) waterbody. Risk is characterized based on the number of 6m docks required for the environmental exposure concentration to exceed a concentration of concern, and whether that number of docks seems reasonable for a 1-ha waterbody. Here, we present how leach rate estimates for acute and chronic aquatic exposures were revised and standardized, and how a reasonable high-end estimate of the number of treated docks was determined, as these metrics currently influence the level of assessed risk. In this presentation we describe the leaching rate units of exposure and leaching study durations that were found to be the best suited for estimating leach rates from pressure-treated, submerged wood. This presentation will also discuss how 2021 satellite imagery was used to calculate a reasonable high-end estimate of the number of docks on a 1-ha waterbody. Lastly, we will discuss how standardized leach metrics were combined with a high-end estimate of the number of docks within a 1-ha waterbody to create a new screening-level model to estimate risks to non-target aquatic organisms from pesticide-treated wood preservatives.

**5.22.V-04 Evaluation of Heavy Metal Content of Canned Fishes and Some Selected Locally Consumed Fishes Sold in Nigerian Market**

***Celestina Ukamaka Ali***[1]*, Grace Otitoju*[2]*, Olawale Otitoju*[2]*, Justina N Chikwendu*[3] *and Joy Nwamarah*[3]*, (1) Nestle Nigeria Plc, (2) Federal University Wukari, Nigeria, (3) University of Nigeria*

Fish is mostly consumed in many parts of the world by human population, as it provides protein of high biological value, essential fats, vitamins and minerals. It is highly perishable commodity and that is why various processing methods are applied to safeguard it for use. There are increased global reports on heavy metals contamination of food samples especially imported fish and this phenomenon has raised lots of concerns among nutritionists and health workers, and environmental toxicologist. Hence, the aim of this research work is to evaluate the amount of heavy metals; Lead (Pb), Cadmium (Cd), Chromium (Cr), Mercury (Hg) and Arsenic (As) present in canned fishes and selected locally fish samples in Nigeria. Heavy metals were determined using UNICAM 939 Atomic Absorption Spectrophotometer at different wavelengths. The results shows that cadmium content was generally low in all the samples ranging from 0.0024 – 0.0099 ppm sample while the local fishes showed the highest amount ranging from 0.0074- 0.0099 ppm. Lead content ranged from 0.017-0.026 ppm. The local fish samples tend to have lower lead levels while the canned fishes had higher level of lead in their tissues. The result also shows that chromium content was generally high in all the samples ranging from 0.037 – 0.058 ppm while the selected fishes show the lowest amount ranging from 0.027- 0.031ppm. Mercury levels were present in some selected fishes ranging from 0.0037 – 0.0046ppm while the canned ones had mercury level in the range of 0.0022-0.0039ppm. The highest mercury level (0.0046±0.01) was observed in sample 3, a locally consumed freshwater fish. The level of arsenic in all the samples ranged from 0.0079-0.015 ppm. Hazard quotient result shows that the HQ was less than 1 (<1) generally for all the samples except for Cr level that was greater than 1 (>1) in sample numbers 11 and 14 with HQ of 1.02 and 1.16 respectively. Therefore, the risk of exposure to majority of the tested samples shows that adverse effects are not likely to occur except for samples number 11 and 14. The levels of these heavy metals in the fish samples were below tolerable and permissible level which may not pose any harm to consumers. However, nutrition education will help to create awareness as well as protecting consumers against heavy metals bioaccumulation in the body.

## Track 6: Engineering, Remediation and Restoration

### 6.01 Assessing and Measuring Effectiveness of Remediation of Contaminated Sediment

**6.01.T-03 Discussion: Assessing and measuring effectiveness of remediation of contaminated sediment**
***Marc Mills**, U.S. Environmental Protection Agency*

**6.01.T-01 Spatial and Temporal Assessment of Biological Effects in Colonial Waterbirds at Contaminated Great Lakes Sites including Areas of Concern**
***Keith Grasman**[1], Lisa Williams[2], Mandy Annis[2], Carly Eakin[2], Donald Tillitt[3] and James Ludwig, (1) Calvin University, (2) U.S. Fish and Wildlife Service, (3) U.S. Geological Survey*
Research over five decades has shown that fish-eating birds of the Great Lakes are excellent sentinel species for assessing and monitoring effects of persistent organic pollutants (POPs), including reproductive problems, deformities, and immune suppression. Two wildlife-related Beneficial Use Impairments (BUIs) at Areas of Concern (AOCs) are recognized by the Great Lakes Water Quality Agreement: 1) bird or animal deformities or reproductive problems and 2) degraded fish and wildlife populations. Current questions under the Great Lakes Restoration Initiative (GLRI) and other federal and state AOC programs include whether these impairments continue and their potential associations with legacy pollutants and contaminants of emerging concern (CECs). In a long-term assessment of birds in the Saginaw Bay and River Raisin AOCs and Grand Traverse Bay, embryonic nonviability (infertility and mortality) in herring gull embryos was 2-3 fold higher than reference sites in both AOCs and Grand Traverse Bay. Deformities associated with polychlorinated biphenyls (PCBs) and dioxins and furans (PCDD/Fs) were observed in embryos and chicks only at contaminated sites. Productivity of 4 week old tern chicks in Saginaw Bay was 35% lower than at reference sites. In the River Raisin AOC, productivity was poor in 7 of 10 years. Breeding herring gull numbers decreased in the River Raisin AOC, and breeding Caspian terns, a state-threatened species, declined in the Saginaw Bay AOC. The phytohemagglutinin skin response (T cell-mediated immunity) was suppressed 50-56% in gull chicks in both AOCs and Grand Traverse Bay, and 49% in terns and 39% in herons in Saginaw Bay. Antibody responses in gull chicks in the River Raisin AOC and Grand Traverse Bay were 1.6-2 fold lower than reference. Time trend analyses showed no significant improvements (and several reductions) in biological endpoints in either AOC or Grand Traverse Bay over the study period. In a shorter spatial assessment of vitamin concentrations in plasma of pre-fledgling double-crested cormorants at 16 colonies, thiamine, vitamin A, and vitamin E were reduced 40-70% at or near Great Lakes AOCs compared to sites outside of or in recovering AOCs. Strong negative associations with contaminants included vitamin A and planar PCBs, vitamin E and PCBs and PAHs, and thiamine and DDE and PCBs. Overall, these biomonitoring programs have demonstrated continuing wildlife BUIs and health effects at AOCs and other contaminated sites.

**6.01.T-02 Successful Use of Tree Swallows to Assess Remedy Effectiveness at Great Lakes Areas of Concern (AOCs) for Bioaccumulative and Non-bioaccumulative Contaminants**
***Christine M. Custer**, Paul M. Dummer and Robert Wesley Flynn, U.S. Geological Survey*
Tree swallows (*Tachycineta bicolor*) are being sampled across the Great Lakes Basin as part of the Great Lakes Restoration Initiative (GLRI) to provide a system-wide assessment of current exposure to contaminants. At selected sites, dredging of contaminated sediment has been done to assist in the removal of associated Beneficial Use Impairments. Tree swallows, because their diet is primarily the aerial stage of benthic aquatic

insects, offer a unique opportunity to assess the effectiveness of sediment removal on contaminant uptake into biota. Changes in the bioavailability of contaminants can be assessed by analysis of eggs and nestlings, and in some cases diet samples, before and after a sediment removal project. While previous remedy effectiveness assessments have been done for polychlorinated biphenyls (PCBs), using the concentration in eggs, concentration in nestling carcasses, and the rate of PCBs accumulated per day in the nestlings, this is not an effective technique for non-bioaccumulative contaminants, such as polycyclic aromatic hydrocarbons (PAHs). To assess changes in PAHs associated with sediment removal, concentration in diet is the preferred metric. Data assessing changes in contaminant exposure at Areas of Concern (AOC) such as the Rouge River and Lower Green Bay and Fox River for PAH contamination, and the Raisin River AOC for PCB contamination will be presented. There was a reduction in PAH exposure post-dredging on the lower Fox River and the Rouge River. There was no decline in PCBs, despite repeated dredging actions, on the Raisin River.

**6.01.T-04 In-Situ Lead Remediation in an Acidic Pine Barrens Wetland**

***Matthew Noerpel***[1]*, Aaron Betts*[1]*, Anna M. Wade*[2]*, Jennifer Goetz*[1]*, Amy Schwarber*[1]*, Stephanie Wilson*[1] *and Todd Luxton*[1]*, (1) U.S. Environmental Protection Agency, (2) Oak Ridge Institute for Science & Education at US EPA, Cincinnati*

Lead remains a common and harmful contaminant at many Superfund and other contaminated waste sites. The Pine Barrens of New Jersey are a large pine forest which dominates the southern half of the state featuring sandy acidic soils. The current study site is located in the northern end of the Pine Barrens with sandy soil over top of a shallow poorly permeable clay layer creating a wetland environment. The site was contaminated with lead pre-1980 and is in the final stages of remediation. As part of the remedy, three separate soil amendment regimes were applied to small test plots in two different regions of the wetlands to assess the effectiveness of the amendments at controlling the mobility and solubility of lead. The first application was a reactive substrate that included biosolids and calcium sulfate addition added to induce the synthesis of galena. The second and third were Apatite II additions to induce the synthesis of pyromorphite. The Apatite II was added both as a surface applied amendment and mixed into the top 6 inches of soil. After approximately 20 and 26 months post application, at the end of the dry and wet seasons respectively, soil and porewater samples were collected and analyzed for a suite of geochemical parameters as well as lead concentration and speciation via X-ray absorption spectroscopy.

The porewater data shows a modest decrease in the lead concentration in the areas with amendment applied as compared to the control areas, however the solid speciation results show limited success of the soil amendments in transforming the lead to the target species. Geochemical parameters such as pH, Eh, cations, anions and TOC were measured and trends with Pb concentration and speciation will be discussed. The reduction in porewater lead and the lack of a change in solid phase lead speciation is likely due in part to lime additions included with the amendments. The increase in sediment pH from the lime may increase lead adsorption on to mineral or organic surfaces. However, the effects from liming cannot be considered a permanent reduction due to the naturally acidic water in the Pine Barrens. This work also highlights the importance of sample handling when a speciation analysis will be performed. Samples collected open to the air and oven dried had a significantly different makeup than those maintained in an anoxic environment for the duration of the experiment.

**6.01.T-05 Comparative Review of Passive Sampling to Conventional Metrics for Evaluating Sediment Remediation Efficacy**

***James S. Grundy***[1]*, Robert M. Burgess*[2] *and Matthew K. Lambert*[2]*, (1) Oak Ridge Institute for Science and Education (ORISE) participant at U.S. Environmental Protection Agency, (2) U.S. Environmental Protection Agency*

Contaminated sediments are present at 36% of active National Priority List sites in the United States, and remediation of these sediments to reduce human health or ecological risk can be costly and complex. Over the last few decades, passive sampling devices (PSDs) have become valuable additions to the toolkit of remedial project managers to improve the characterization of contaminant transport and assessment of risk at sediment sites, and to evaluate the effectiveness of remedial actions.

This study has two aims: (1) to identify sites where PSDs have been used to support cleanup efforts, and (2) assess how PSD-derived remedial endpoints compared to conventional metrics. To do so, this study comprehensively reviewed literature on PSD use during the sediment remediation process, from feasibility studies to monitoring remedy effectiveness. Passive samplers have been used to support sediment remediation projects at nearly 100 sites globally resulting in over 130 peer-reviewed scientific publications and numerous reports and conference presentations. Generally, there were four categories of use for PSDs to support remedial investigations: (1) freely dissolved concentrations, (2) site-specific partition coefficients, (3) bioaccumulation evaluations, and (4) mass flux estimates between site media. Most studies focused on hydrophobic organic contaminants, namely PAHs and PCBs; roughly 20% of studies used diffusive gradient in thin film (DGT) devices for heavy metals, indicating these tools are often utilized when this class of contaminants are of concern.

Of the peer-reviewed literature, about 1/3 of articles solely used PSDs to determine remedial efficacy, highlighting the advances, utility, and growing acceptance of polymeric PSDs. In the other articles, the most common conventional metrics investigated alongside PSDs were bioaccumulation (33%), pore water grab samples (16%), toxicity (15%), and water column grab samples (15%). Generally, remedial efficacy investigated through PSD endpoints correlated with conventional methods, but poor agreement was found in some cases (e.g., when PSDs sampled different sediment depths than what biota were exposed to). Results of this review support the continued and potential expanded use of PSDs for a range of applications supporting decision-making at sites undergoing remediation.

**6.01.T-06 The effects of sorption kinetics of PCBs on powdered and granular activated carbon on in-situ sediment treatment and capping**

***Danny Reible***[1], *Xiaolong Shen*[1], *Tariq Hussain*[1], *Micala Mitchek*[2] *and Joe Wong*[2], *(1) Texas Tech University, (2) ADA Carbon Solutions, Inc*

Activated carbon (AC) has been applied widely in water treatment as a strong sorbent for organic contaminants and in in-situ treatment and capping for managing legacy contaminants in sediments. Commercial AC is normally available in various particle sizes ranging from 0.02 mm to 5 mm, and this may lead to significant differences in transient adsorption performance in applications. This may be particularly important for high molecular weight sediment contaminants such as PCBs, whose molecular size is similar to a significant fraction of the pores in activated carbon. The kinetics of uptake is also difficult to measure due to the extremely low aqueous equilibrium concentration of such compounds.

In this study, batch experiments were conducted to measure PCBs adsorption kinetics on granular and powdered AC and polydimethylsiloxane (PDMS) fibers to measure aqueous concentrations over time. The experiment was conducted in glass containers loaded with water at known PCB concentration and containing 10 mg/L natural organic matter (NOM) and activated carbon. Blanks without activated carbon were used to estimate kinetics and equilibrium uptake to PDMS and NOM using measured PDMS loading and a first-order kinetic model. The PDMS in slurries with activated carbon was then used to estimate kinetics and equilibrium uptake of the various PCBs onto the activated carbon. A first-order kinetic model and an intraparticle diffusion model were established to describe the kinetic sorption of PCBs in the water-AC-PDMS system. The coefficients evaluated from the two models are compared to literature values from both batch experiments and column studies. A correlation between the rate coefficients and the particle size of AC is established. For example, the half-life for sorption of PCB 101 in the powdered carbon (0.02mm) is approximately 5 weeks but about 20-40 weeks is required for granular carbon (1.1. mm). The surrounding aqueous concentration can decrease much more rapidly depending upon the AC to water ratio. The measured kinetics was used to predict AC performance in various scenarios of sediment in-situ treatment and amended capping.

This study demonstrates the usage of polymeric passive samplers in exploring the sorption kinetics for low solubility compounds in AC and guides predictions of the sorption kinetics of PCBs in sediment remedies. Slow granular AC kinetics can significantly limit performance in low residence time conditions.

**6.01.T-07 Assessing Effectiveness of Management Options in the Anacostia River by Linking PCB Fate and Transport with Bioaccumulation Model**

*Mandar Bokare[1], Nathalie Lombard[1], Alfred Pinkney[2], Dev Murali[3] and **Upal Ghosh**[1], (1) University of Maryland, Baltimore County, (2) U.S. Fish and Wildlife Service, (3) Department of Energy and Environment, Canada*

Polychlorinated biphenyls (PCBs) are major contaminants of concern in the Anacostia River, and are responsible for fish-consumption advisories in the District of Columbia (DC). Uptake of PCBs in the aquatic food web is governed by the dissolved concentration in surface water and sediments. We recently performed measurements of surface water, sediment porewater, and air-phase PCB concentrations using polyethylene passive samplers to provide the first accurate mass balance of dissolved PCBs in the Anacostia River. This mass balance enabled identification of a major tributary and contaminated sediment hot-spots as the sources having the most negative impact on the water body. In the present study, hydrological data from an existing hydrodynamic model was integrated with results from the mass balance study and used to simulate the concentration of individual PCB congeners in surface water and sediments in six segments of the Anacostia River. Results were validated against measured data and the model was used to simulate impact of remediation scenarios such as natural recovery, source control, and sediment remediation on PCB concentrations in the Anacostia River. Model outputs were linked to a validated aquatic food-web model to predict the quantitative impact of remediation strategies on PCB concentrations in fish. Results demonstrate the critical need for controlling ongoing source from a polluted tributary to realize the benefits of focused remediation of sediment hot-spots. Overall, the study demonstrates the utility of developing mechanistic models to anticipate recovery after remedial actions. The predictive framework developed in this study can improve the remedial decision-making process at other contaminated sites.

**6.01.T-08 Sources of Polychlorinated Biphenyls to Upper Hudson River Fish and Dredging Effectiveness**

*Braina Stahl[1], Staci L. Capozzi[2], Mohson Al Hello[3], Kelly L. Francisco[3], Marcia S. Meixler[3] and **Lisa A. Rodenburg**[3], (1) Rensselaer Polytechnic Institute, (2) Indiana University, Bloomington, (3) Rutgers University*

Polychlorinated biphenyls (PCBs) are persistent, bioaccumulative, and toxic chemicals that are the dominant contaminant in the Upper Hudson River (UHR) in New York State where two General Electric (GE) plants historically discharged PCBs to the river. Portions of the UHR were dredged from 2009-2015 to address PCB contamination. In 2017, the first post-dredging survey of yearling feeder fish and sediment PCB contamination was conducted to establish a baseline for the recovery of the river. Prior analysis of the sediment data from the 2017 survey indicated that ~2% of the PCBs in the surface sediment were higher in molecular weight than the main formulation used by GE and therefore arose from non-GE sources. In this work, the fish PCB data from the 2017 survey were analyzed using Positive Matrix Factorization (PMF). Empirical Bayesian Kriging was used to estimate PCB concentrations in the sediment at the locations where fish were collected. The results suggest that PCBs that are the products of microbial dechlorination bioaccumulate in the fish. Further, the results suggest that some of the PCBs in the fish may have come from non-GE sources. The proportion of non-GE PCBs in the fish is higher than their fraction in the sediment, but can be explained by the higher molecular weight of the non-GE mixture which causes it to bioaccumulate more effectively than GE PCBs.

**6.01.P Assessing and Measuring Effectiveness of Remediation of Contaminated Sediment**

**6.01.P-We177 Macroinvertebrate Bioassessment of Great Lakes Area of Concern (AOC) Sites: Choosing the Best Metrics and Analyses to Assess the Success of Remediation and Restoration Efforts.**

***Roger Yeardley****, Marc Mills, Michael Bruce Griffith and James M. Lazorchak, U.S. Environmental Protection Agency*

The U.S. EPA's Office of Research and Development (ORD), in working with its multiple state and federal partners, is tasked with helping to determine if conditions have improved post-remediation at Great Lakes Area of Concern (AOC) sites. For one core line of evidence, macroinvertebrate communities, some state environmental agency partners have standard indices which have been historically used for their ongoing bioassessments and delisting of Beneficial Use Impairments (BUIs) at AOC sites. For example, New York State Department of Environmental Conservation (NYSDEC) has a multi-metric Biological Assessment Profile (BAP) index and Ohio EPA uses a 10-metric index called the Lacustrine Index of Community Integrity (LICI) for bioassessment of water quality with macroinvertebrate communities.

However, the overarching goal is to report whether there is a change in communities that reflects a change in water quality, pre- and post-remedy. As a research organization with a core skill of methods development, one of the contributions that ORD can make towards this goal is to explore what types of metrics and analyses offer the best chance to detect differences in communities. The goal to detect community differences is complicated by the lack of suitable reference sites at most AOCs, which are in highly populated and developed areas.

Our presentation will explore what macroinvertebrate community metrics Great Lakes states are finding useful, as well as analysis of the usefulness of some common and not-so-common metrics and analyses with data from multiple AOC sites. We will also touch on how to deal with the reference site issue. One category of analyses which appears to have a high power to detect differences in macroinvertebrate communities is multivariate ordinations. For example, ANOVAs comparing total taxa richness at 8 Niagara River tributary sites showed no significant differences among sites for communities collected with either the NYSDEC ($p=0.667$) or US. EPA multi-plate sampler type ($P=0.093$). However, Nonmetric Multidimensional Scaling (NMS) followed by Multi-Response Permutation Procedure (MRPP) found much more significant differences in community type between sites ($T=-14.53$, $p=0.000000001$) than between sampler type ($T= -5.25$, $p=0.000272$).

**6.01.P-We178 Evaluation of in-situ sand capping as a second aid to alleviate the impact of phenol spill to the aquatic environment**

***Yongju Choi*** *and Aleum Lee, Seoul National University, Korea, Republic of (South)*

Studies on sediment remediation techniques have been targeted on contaminants that show evident persistence in sediment (e.g., hydrophobic organic compounds, heavy metals). However, residual sediment contamination has also been found for chemicals with moderate hydrophobicity and low volatility such as phenol. In case when massive slug of such chemicals enters the aquatic environment, e.g., via chemical spill accident, sediment may remain under impact for months to years, acting as a source of the contaminants to the aquatic food chain. In this study, we evaluated the feasibility of using in-situ sand capping as a measure of mitigating the release of sediment phenol to the water column (so-called "second aid") after applying "first aids" against accidental chemical spill of the contaminant. Sediment near an estuarine industrial complex in Korea was collected to evaluate their physical properties, phenol sorption characteristics, and biodegradation rate of phenol, which were parameterized as input variables for CapSim, a software that predicts the performance of sediment capping under various field scenarios. The CapSim simulation demonstrated that the performance of sand cap to prevent the release of phenol to the water column was a function of cap thickness, pore-water movement, and biodegradation rate. These factors showed intimate interplay among one another to determine the performance. For example, sand cap with minimal depth (30 cm or less) was predicted to reduce the total mass of phenol release to the water column by several orders of magnitude if phenol was degraded at a rate of 0.0565/day and upward pore-water movement was absent: under this scenario phenol was expected to be almost fully degraded until it could pass the sand cap via molecular diffusion to reach the water column.

**6.01.P-We179 Assessment of Dechlorination Rate and Enantioselectivity of Reductive Dehalogenase Exposed to Metals**

***Catherine Sumner** and Cindy Lee, Clemson University*

The microbial reductive dehalogenation of polychlorinated biphenyls (PCBs) can be an important step to remediate regions contaminated with the persistent organic pollutant. Several researchers in recent years have identified reductive dehalogenases (RDase), one enzymatic pathway responsible for dechlorination of PCBs. However, the mechanisms underlying the enantioselective transformation of chiral PCBs are unexplored. Enantiomers are one of two stereoisomers that are non-superimposable mirror images of each other. Field samples from around the world indicate non-racemic enantiomeric fractions of PCBs, demonstrating that one enantiomer is in higher concentration than the other. Commercial PCBs were released into the environment at racemic levels, so the observations propose the question as to how chiral PCBs degraded enantioselectively. A few enzymatic pathways in RDases have been only recently explored, but the question involving what step or steps in the dehalogenation is enantioselective has not been determined as of yet. There are several components, such as cofactors, that can promote enzymatic functions affecting enantioselectivity. One recent study showed that hazardous metals inhibited RDase function, implying that metals play a role in the enzymes' pathway. Metals have also been shown to serve as electron donors or acceptors and can form ligands that interact with enzymes. We are currently exploring how naturally occurring, essential metals such as zinc and copper affect the dechlorination rate of trichloroethene (TCE) and PCBs. TCE in the project is used as a halo-primer to catalyze the dechlorination of PCBs. This research is being conducted using lab microcosms from contaminated sediment cores collected from the watershed of Lake Hartwell, SC, USA. This Superfund site is ideal for this prospective research because PCBs were the major contaminants with only minor amounts of chlorinated solvents. Previous research by our group confirmed enantioselective degradation was occurring in the sediments. From when the plant was opened in 1955 to its closure in 1977, it was estimated that over 220 tons of PCBs were released into Town Creek that feeds into Lake Hartwell. We will present an experimental design of our study to discover the relationships among essential metals and enantioselective dehalogenation along with preliminary data.

**6.01.P-We180 Innovative Blending of Large Volume Dredged Materials to Reduce Pollutant Risk and Enable Sustainable Reuse**

***Upal Ghosh**, Songjing Yan and Louis Cheung, University of Maryland, Baltimore County*

Sediment from Baltimore Harbor has been reported to have a range of pollutants, including PCBs, PAHs, organochlorine pesticides, and heavy metals. However, the full extent of pollutant bioavailability and risk posed by the dredged sediments originating from maintenance dredging of the Baltimore Harbor channels is not fully understood. Several large volumes of industrial byproducts such as biochars made from agricultural waste and biosolids have shown unique sorption characteristics and can be explored as low-cost amendments for contaminated sediments. These large volume byproducts are especially attractive from the standpoint of sustainable utilization of material resources and synergistic linking of two waste products to achieve an end product that poses less risk and is suitable for beneficial reuse such as in wetland creation. In this research, we evaluated sorption characteristics of six industrial byproduct biochars and explored the idea of pollutant bioavailability reduction using these amendments to the contaminated sediments.

The primary objective of this research is to develop a rational framework to design amendment choice and dosing to enable pollutant sequestration in dredged sediments. The following objectives have been addressed: 1) selection and characterization of study sediments; 2) selection, procurement, and characterization of potential amendments; 3) determination of optimal amendment and dose for treatment of dredged sediments for risk reduction. The biochars sustainably produced from cow, poultry, mushroom compost, biosolids, as well as activated cow and poultry biochars were analyzed as potential additives for the amendment, along with activated carbons. Sorption isotherms were measured for the selected organic contaminants and compared with literature values. The biochar sorption capacity is 2-3 orders of magnitude lower than the activated carbons. Top

3 selections of biochars with 3 doses (1%, 3%, 5%) and 2 selections of activated carbons with 1% dose were used to perform *ex situ* experiments with dredged sediment samples in the laboratory.

This research studied the effectiveness of amendment mixtures/composites on sediment samples obtained from past dredging activities in Baltimore Harbor containing complex mixtures of PAHs, PCBs, and pesticides. With the optimal choice of amendments, this research is expected to provide a low-cost alternative for sustainable reuse of large volumes of byproducts generated from various sources.

**6.01.P-We181 Riparian Spiders: Biosentinels of Polychlorinated Dibenzo-p-dioxin and Dibenzofuran Contaminated Sediment**

***Gale B. Beaubien***[1]*, Dalon White*[1]*, Marc Mills*[1]*, David Walters*[2]*, Ryan R. Otter*[3]*, Kenneth Fritz*[1] *and Brian Crone*[1]*, (1) U.S. Environmental Protection Agency, (2) U.S. Geological Survey, (3) Middle Tennessee State University*

Polychlorinated dibenzo-p-dioxin and polychlorinated dibenzofuran (PCDD/F) are persistent, toxic, and bioaccumulative. Currently, PCDD/F monitoring programs primarily use organisms with large home ranges to monitor temporal trends over a broad spatial scale (e.g., fish, birds), but biosentinels that provide targeted sediment contaminant information across a small geographic area have yet to be developed. Recent studies have demonstrated that riparian orb-weaving spiders, specifically those that have a small home range (meters) and feed primarily on adult aquatic insects, can indicate the source and magnitude of sediments contaminated with dioxin-like chlorinated compounds, such as polychlorinated biphenyls (PCBs). In the present study, our overall aim was to understand the utility of riparian spiders as biosentinels of PCDD/F contaminated sediments. We collected surface sediment and spiders within the St. Louis River Watershed Great Lakes Region; near Duluth, MN, USA) and analyzed each for homologue and total PCDD/F concentrations. We tested whether concentrations and homologue profiles in sediments were similar to those observed in spiders. We also tested whether spider contaminant concentrations were significantly correlated to surface sediment contaminants across a broad sediment contamination gradient. Total PCDD/F sediment concentrations (mean ± std. error: 286,591 ± 97,614 pg/g) were significantly higher than riparian spiders (2,463 ± 977 pg/g). However, sediment and spider relative abundance profiles were not significantly different, and homologue concentrations in spiders were significantly and positively correlated with surface sediment ($R^2 = 0.47$, $p < 0.001$). These results indicate that riparian orb-weaving spiders may be used as sentinels of ambient environmental concentrations of PCDD/F in contaminated sediments of freshwater ecosystems.

**6.01.P-We182 Characterizing Contaminated Sediments in Lower Maumee River, Maumee Area of Concern Ohio, in Order to Support Remedial Alternative Development for Removing Beneficial Use Impairments**

***Karen G. Keil***[1]*, Marc Mills*[2]*, Victoria Harms*[1]*, Ashley R. Binion-Zuccaro*[1]*, Vanessa Steigerwald Dick*[3]*, Lynn Ackerson*[3] *and Susan Virgilio*[2]*, (1) U.S. Army Corps of Engineers, (2) U.S. Environmental Protection Agency, (3) Ohio Environmental Protection Agency*

The 130-mile-long Maumee River originates near Fort Wayne, Indiana, and the lower 23 miles flowing through Toledo, Ohio is included within the Maumee Area of Concern (AOC). It includes a 7-mile-long federally maintained navigation channel for the Port of Toledo. The river's industrial and commercial uses have left a legacy of contaminated sediments which may be contributing to beneficial use impairments including degradation of benthos and restrictions on dredging. Several sediment characterization efforts have been conducted in the Maumee River since 2011 and have identified 2 distinct areas of sediment contamination. An additional sediment investigation was conducted in 2021 with the goal of obtaining sufficient data to develop sediment remedial alternatives supporting removal of beneficial use impairments. Locations of interest were grouped into sub-areas based on the level of sediment contamination previously identified. Data Quality Objectives and the associated sampling and analyses varied in each area. In areas previously identified as contaminated, surface sediment samples were collected to better characterize the toxicological and bioaccumulative (biological) nature of contamination. The results from these types of analyses will inform the relationship between sediment contamination and beneficial use impairments, especially when considered in conjunction with porewater analysis and biological community surveys performed within the same areas of the river. At the outer portions of areas potentially contaminated, sampling efforts focused on delineating the extent of sediment contamination by collecting a higher density of sediment core samples for chemical analyses only. This investigation confirmed the nature and extent of an area of polyaromatic hydrocarbon sediment contamination which likely contributes to the degradation of benthos beneficial use impairment. A second area of the river in which some natural attenuation of polychlorinated biphenyl contamination may be occurring was also characterized. A Feasibility Study is being conducted to formulate potential remedial alternatives for both these areas of the Lower Maumee River, leading to sediment management actions supporting removal of beneficial use impairments. The results from this sediment investigation will also support baseline characterization for future remedy effectiveness assessments once management actions are complete.

## 6.02.P Contaminated Sediment Toxicity, Risk Assessment and Management, Remediation, Restoration, Climate Change Resiliency

### 6.02.P-Mo167 Evaluating toxicity of sediments from the Little Calumet River, Indiana

***Nile E. Kemble***[1], *Jeffery Steevens*[1] *and Daniel Sparks*[2], *(1) U.S. Geological Survey, (2) U.S. Fish and Wildlife Service*

The eastern portion of the Little Calumet River is located in Porter County, Indiana and flows through Indiana Dunes National Park and before entering Lake Michigan. The purpose of this study was to evaluate sediments from the Little Calumet River for potential chemical contamination and associated aquatic toxicity. Ammonia and metals have been identified as potential contaminants of concern. We examined a total of 12 sites along the Little Calumet River that included three reference sites that were selected based on previous studies. Samples were collected by ponar, homogenized in the field, and subsampled for sediment chemistry, porewater analysis, and sediment toxicity tests. Toxicity tests were conducted following standard methods for acute and chronic sediment toxicity tests with freshwater invertebrates. Species tested included the amphipod *Hyalella azteca* (28-d exposure), the midge *Chironomus dilutus* (10-d exposure), and the mussel *Lampsilis siliquoidea* (fatmucket; 28-d exposure). These three species were selected because they have different exposure pathways and contaminant sensitivities. Toxicity test endpoints included survival, growth, and biomass. Mean control survival at the end of the exposures met test acceptability criteria for all three species. Results of these studies found significant reductions in survival and growth. Results of the bioassays will be compared to chemical concentrations in sediment and porewater as well as benthic survey data. This study will be used to inform resource managers on the potential biological effects of contaminants in this river system.

### 6.02.P-Mo169 The Presence, Distribution, and Concentration of Trace Metals in Surface Waters and Sediments Collected Near a Virginia Coal Ash Repository

***Elizabeth Tyler***, *Rachael Harrington, Catherine Crowell, Leanna Giancarlo, Ben Odhiambo Kisila and Tyler Edward Frankel, University of Mary Washington*

The Chesapeake Bay watershed contains several coal-burning power stations located along its waterways. Coal ash, one of the largest forms of industrial waste, is primarily produced by power stations and disposed of in coal ash repositories. Coal as is also known to be heavily enriched with trace metals and these contaminants are then able to enter surrounding aquatic environments. Few studies have examined trace metal contamination within the Potomac-Shenandoah watershed stemming from these repositories. Thus, the goal of this study was to assess the spatial and temporal distribution of trace metals in sediments and surface waters adjacent to the Possum Point power station (Quantico, VA). Water and sediment samples (grab and core) were collected from several sites upstream, midstream, and downstream from the station. Trace metals from each sample were extracted and analyzed using ICP-OES (inductively coupled plasma-optical emission spectroscopy) for the presence and concentration of Al, As, Cd, Ca, Cr, Cu, Fe, Pb, Mg, Mn, Se, and Zn. Cores were sectioned at 2-

centimeter intervals and sediment chronology established using $^{210}Pb$. While this study is still ongoing, we expect to find elevated concentrations of these metals midstream and downstream from the power station. Based on chronological data, we also expect to observe enriched trace metal deposits that occur after the coal ash repositories were created. This study will provide vital information regarding the prospective impacts of coal-burning repositories on the release and mobilization of trace metal contaminants within aquatic ecosystems in the Chesapeake Bay region.

### 6.02.P-Mo170 Validation of a Novel Test System for Exposure Assessment With Benthic Invertebrates and Chemical Activity as a Dose Metric

***Sebastian Abel***[1]*, Sophie Steigerwald*[1]*, Gastón Alurralde*[1]*, Ann-Kristin Eriksson-Wiklund*[1]*, Elena Gorokhova*[1] *and Jarkko Akkanen*[2]*, (1) Stockholm University, Sweden, (2) University of Eastern Finland, Finland*

Assessing the risk of sediment-associated hydrophobic organic contaminants (HOCs) relies on the experimentally determined effective concentrations (e.g. $EC_{50}$) of individual compounds. Due to the vastly different physicochemical properties of HOCs, as well as the sorption strength being dependent on the sediment matrix, these concentrations can be misleading. To account for these factors, an approach based on chemical activity, or the closely related freely dissolved concentration ($C_{free}$) can be used. Indeed, both bioavailability and mobility of a chemical are strongly dependent on its chemical activity rather than bulk sediment concentration. Here, we demonstrate that the chemical activity provides a useful and comparable metric for evaluating dose-response relationships in benthic invertebrates exposed to HOCs. The invertebrates are common test species representing different feeding mode and burrowing behaviors: *Lumbriculus variegatus*, a deep-dwelling oligochaete, *Chironomus riparius*, a shallow-burrowing chironomid larva, and amphipod *Hyalella azteca*, a suspension-feeder inhabiting sediment surface. As model-HOC mixture, 4 PAHs (Acenaphthene, Fluorene, Phenanthrene and Fluoranthene) are loaded into the sediment through saturated, aqueous solution to obtain a target chemical activity. Throughout the loading process, the solution is constantly replenished to avoid HOC-depletion. This loading and exposure methodology ensures the predictability of the contaminants' bioavailability within the system. Following the exposure times of 28 d for *L. variegatus*, 12 d for *C. riparius* and 14 d for *H. azteca*, the adverse effects of the PAH mixture on organism growth, survival, and oxidative stress biomarkers will be assessed. The observed effects will be compared to internal (bioaccumulated) concentrations of PAHs and used to calculate effective activities ($Ea_{50}$) that will contribute to the recommendations for improving the bioassay methodology.

### 6.02.P-Mo171 Spatial Analysis of Dam Sediment and Metal Contamination in the Northern Nashua River Basin

***Jack Taylor Lanier***[1]*, Elyse V. Clark*[2] *and Ben K. Odhiambo*[1]*, (1) University of Mary Washington, (2) Fitchburg State University*

Fitchburg, Massachusetts is a heavily industrialized city with over 20 dams located within the North Nashua River basin. Many of the dams, which were constructed in the mid- to late-1800's, are abandoned. Metal fluxes and accumulation in fluvial ecosystems reflect natural weathering and associated sediment fluxes from the basin; but in most cases this process is exacerbated by the progressive anthropogenic land-use intensification with human population increases. It is well established that sediments trapped behind dams are a sink for contaminants from various land uses, known point sources, and atmospheric deposition. Therefore, spatial analysis of sediments behind multiple dams at high resolution in a single basin enables characterization of the dynamics between modern urbanization and legacy industry. This study seeks to characterize the spatial variability of metal concentrations in sediments behind multiple dams on the North Nashua River in order to infer the impact of land use and legacy industry on fluvial systems. Data collection consisted of multiple sediment cores and grab samples from seven dams in upstream to downstream locations along the North Nashua River. Samples were prepared using the aqua regia digestion method (USEPA 3050B) and were analyzed using an inductively coupled plasma mass spectrometer (ICP-MS) to determine metal concentrations in the surficial and core sediments of North Nashua River Basin. Preliminary results from trace metals enrichment analysis indicate significant to extreme enrichment of arsenic, cadmium, and lead, and low enrichment of copper and zinc. Specifically, As ranges from 1-15, Cd 1-250, Pb 1.5-15, Cu 0.1-6, and Zn 0.9-11. Initial hotspot analysis shows the greatest concentrations of metals in the south-central region of the study area; located adjacent to the town of Fitchburg and at the confluence of several sub-basins into the North Nashua River. Further Principal Component Analysis and other statistical analyses will elucidate the role of legacy sediments and contemporary land-use intensification in influencing metal distribution and loading in the North Nashua River Basin. This knowledge will provide a base for future research relating to contamination from the region's industrial legacy and aid in any potential future remediation activities.

**6.02.P-Mo172 Target Lipid Model and Empirical Koc Values to Predict PCB Sediment Toxicity to Invertebrates: Model Development & Aqueous Toxicity Data**

***Phyllis Fuchsman****, Kyle Fetters and Alison O'Connor, Ramboll*

Quantifying causal exposure-response relationships for PCB toxicity to benthic invertebrates can be an important component of contaminated sediment assessments, informing cleanup decisions and natural resource injury determinations. Building on prior analyses, we demonstrate that the target lipid model (McGrath et al. 2018, ETC 37:1579) accurately predicts aquatic toxicity of PCBs to invertebrates, as invertebrates are not susceptible to the "dioxin-like" mechanism of toxicity. The target lipid model provides a means to account for effects of PCB mixture composition on the toxicity of bioavailable PCBs. We also incorporate updated data on PCB partitioning between particles and interstitial water in field-collected sediments, to better account for effects of PCB mixture composition on PCB bioavailability. Because organic carbon-water partition coefficients ($K_{OC}$ values) vary widely across sediments, we identify low-end, central-tendency, and high-end $K_{OC}$ inputs for PCB congeners and homolog groups. Spiked sediment toxicity data and case studies of PCB-contaminated sediment sites support the model's predictions. The updated model should provide a useful tool for both screening-level and in-depth risk analyses for PCBs in sediment, and it should aid in diagnosing potential contributing factors at sites where sediment toxicity and benthic community impairment are observed.

**6.02.P-Mo173 Target Lipid Model and Empirical Koc Values to Predict PCB Sediment Toxicity to Invertebrates: Spiked Sediment & Case Study Data**

***Kyle Fetters****, Phyllis Fuchsman and Alison O'Connor, Ramboll*

We applied the target lipid model to predict PCB toxicity to benthic invertebrates, using empirical organic carbon-water partition coefficients ($K_{OC}$ values) to extend aqueous toxicity predictions to sediments. Model predictions compared favorably to sediment toxicity data from spiked sediment toxicity studies and case studies at PCB-contaminated sediment sites. We identified only two published spiked sediment toxicity studies with PCBs that used appropriate sediment equilibration procedures, and these studies showed no toxicity at the highest tested concentrations. Case studies provided a wider range of biological responses for model validation. Case studies were included if: (1) PCB mixture composition was characterized; (2) toxicity was tested with whole-sediment exposures and/or benthic community composition was evaluated; (3) PCBs were the major sediment contaminants known from the site, or the role of other contaminants could be adequately evaluated; and (4) data quality and documentation were adequate. Primary case studies included Upper Hudson River, Anniston PCB Site, New Bedford Harbor, and Saglek Bay. Supplemental case studies, with a potentially larger role of co-contaminants, included Ashtabula River and Brunswick Estuary. Toxicity thresholds expressed as summed toxic units always exceeded 1, as predicted. Also, predicted differences in toxicity among PCB mixtures (e.g., relatively low toxicity of Aroclor 1268) were supported by case study results.

**6.02.P-Mo174 A Framework for Evaluating Legacy Sediment Quality Behind Dams to Prioritize Proactive Sediment Assessment and Management**

***Gary R. Long****, Justine Decker, Samuel Parker and Nigel Goulding, EHS Support LLC*

More than 80 percent of the approximately 90,000 dams listed in the U.S. Army Corps of Engineers National Inventory of Dams (NID) will exceed their 50-year engineered life expectancy by 2030, creating potential hazards to life and property in the event of catastrophic failure. Environmental impacts of aging dams are associated with the loss of ecological functions (e.g., fish passage) and the potential downstream release of contaminated sediments accumulated behind dams following a failure. Improper management of impounded sediment may lead to a downstream release of sediment-related contaminants far afield from the dam in the event of a failure or an extreme hydrologic event during dam removal. Given the potential environmental liability associated with a release of contaminated sediments, the proactive assessment and management of accumulated sediments prior to a dam failure or removal effort will significantly reduce remediation costs for dam owners or potentially responsible parties (PRPs) compared to remediation following a release.

This work describes the development of a geospatial database and a framework to inform and prioritize proactive sediment assessment and management for dam owners or PRPs associated with the accumulation of contaminated sediments behind dams and the potential uplift of restoration projects. We created a geographic information system (GIS)-based platform to incorporate spatial data and attribute information for dams at a watershed scale. We then developed a decision framework to assign categorial risk rankings for dams based on the likelihood of contaminated sediment accumulation as a function of catchment attributes. Using this framework, we derived dam risk rankings to inform the need for further assessment of sediment quality or dam structural integrity to fully evaluate the potential for contaminated sediment releases. This framework and supporting database will benefit government agencies, dam owners, or PRPs that contributed to condition of legacy sediments behind dams by allowing them to identify, prioritize, and proactively assess and manage sediment liabilities to mitigate the potential for downstream release.

## 6.02A Contaminated Sediment Toxicity, Risk Assessment and Management, Remediation, Restoration, Climate Change Resiliency

### 6.02A.T-01 Integrating Restoration into Remedial Design for a Tidal Wetland

*Sarah Greenfield[1], **Benjamin Johnson**[2] and Jenna DiMarzio[2], (1) Oregon Department of Environmental Quality, (2) GSI Water Solutions, Inc.*

Considering habitat restoration and mitigation opportunities early in the cleanup process at contaminated sites can help support development of remedial design alternatives that address environmental risk and restore ecological function. In St. Helens, Oregon, the Oregon Department of Environmental Quality is applying this approach at a legacy cleanup site. This work will help design professionals better understand conditions that influence wetland habitat types and the fate and transport of contaminants.

Historical operations at the site released arsenic, mercury, and organic contaminants to the adjacent wetlands of Scappoose Bay. Mudflats and braided channels traverse the site and are tidally influenced by the Columbia River. The area supports a diverse community of emergent wetland and forested riparian vegetation, including culturally significant plant species like the wapato. These emergent wetlands provide important habitat cover and food sources for native mussels, amphibians, lamprey, and fish. Wetland habitat types at the site vary in surface elevations and the corresponding inundation frequency associated with tidal fluctuations and river stage. Inundation frequency also influences the fate and transport of inorganic contaminants (arsenic and mercury) throughout the site by creating variability in reducing conditions that can alter the speciation and mobility.

The mobility of inorganics is being investigated to assess habitat distributions, redox conditions, and the relationships between inundation frequency and habitat type. Topographic surveys were conducted to establish high-resolution surface elevations and complete a terrain model for the site. Habitat surveys will characterize different habitat types and tie them to elevations derived from the surveys. Stilling wells outfitted with pressure transducers were installed throughout the site's channel networks to provide insight into temporal and spatial patterns of inundation. The mobility and speciation of inorganics are being assessed using passive sampling techniques, in situ redox profiles, and geochemical cores. The results will be used to develop remedial design alternatives that address contamination, result in post-construction surface elevations conducive to re-establishing natural habitats, and limit impacts to existing critical wetland habitats.

**6.02A.T-02 Implementing a Resilient Wetland Remediation Project Considering Elevated Climate Activity**

*Lucas Hellerich, **Nicholas Hastings**, Jared Port, Bill DePascale and Jack Markey, Woodard & Curran*

In the Northeast US, a forested, riparian wetland impacted with nickel over approximately 2.3 acres, required remediation to mitigate potential ecological risk. The area requiring remediation was primarily within the FEMA Floodway and 100- and 500-Year Flood zones. Further, the stream situated adjacent to the wetland has a mostly developed watershed, and it experiences significant changes in flows and water elevation during storms.

The remedy was implemented in 2021 and consisted of removal of soil and sediment containing nickel above ecological risk-based criteria, followed by wetland and habitat restoration. Anticipating the potential for stormwater runoff and flooding of the stream, planning and contingencies were incorporated into the project. Prior to the remediation phase, the excavation limits were refined through higher resolution soil and sediment sampling. The resulting excavation consisted of a central two-foot-deep area and two six-inch-deep areas. This refinement facilitated regulatory approval of not requiring post-removal verification samples, critical to managing risks during a soil removal and backfilling program.

Resiliency was a major consideration during project design and planning. The design included robust flood control and erosion control measures, backfill soils and wetland tree and shrub species appropriate for future flooding conditions, and an improved permanent stormwater outfall structure. A comprehensive contingency plan was established. Moreover, large trees were to be preserved within the excavation areas to provide long-term soil stabilization, mitigate erosion of the stream's shoreline, and provide a canopy to improve the survivability of planted trees and shrubs.

Three major storms (a hurricane and two tropical storms) passed over the project during the active remediation phase of the project. The contingency plan was followed to mitigate impacts from the storms. The project area was mostly inundated twice; however, scouring of newly restored areas and other impacts were limited. First year restoration monitoring results will be reviewed. Lessons learned will be shared.

**6.02A.T-04 Evaluation of Relevant Exposure Pathways in a Unique Environmental Setting within a Large Dynamic River System**

***Jennifer L. Peterson** and Michael Poulsen, Oregon Department of Environmental Quality*

Bradford Island is a recently listed Superfund site located in the Pacific Northwest upstream of Bonneville Dam on the Columbia River, the fourth largest river in the United States by volume. Disposal practices on the island resulted in contamination of the river from the direct disposal of electrical equipment containing polychlorinated biphenyl (PCB) non-aqueous phase liquids, such as capacitors, light ballasts, and insulators. Electrical equipment was removed from the river in 2000 and 2002, and contaminated sediment was removed by diver-directed dredging in 2007. Despite these remedial actions, fish and shellfish sampling in 2011 continued to show significant levels of contamination, with PCB concentrations up to 183 mg/kg in smallmouth bass tissue. A fish consumption advisory was issued. Four tribes have treaty rights to fish in the area, and there are established fishing platforms in the immediate vicinity of Bradford Island. The Columbia River is listed for several state and federally listed fish species. Determining the nature and extent of remaining PCBs in the aquatic environment is therefore a priority. The dynamic nature of the environment includes a river bottom dominated by boulders and cobblestones as compared to sediment, significant surface water velocity around the island, and reversals in water flow direction determined by spillway operations. This necessitated a focus on site-specific collection of fish and shellfish tissue for exposure assessment and monitoring, or an appropriate surrogate via passive sampling, rather than a focus on sediment concentrations. Exposure pathway assessment tools and results will be presented.

**6.02A.T-05 Evaluating Human Health Risks from Exposures to Impacted Sediment in Risk Assessments**

***Mayble Abraham*** *and Chrissy Peterson, EHS Support LLC*

Humans can be exposed to sediments impacted with chemicals during recreational activities, in occupational settings (e.g., dredging), and through subsistence fishing. Risk assessments evaluate human exposure to impacted sediments and identify potential for excess adverse health outcomes as a result. This presentation will walk through the four steps of a risk assessment (i.e., hazard identification, exposure assessment, toxicity assessment, and risk characterization) in the context of evaluating sediment exposures and discuss nuances of the process as it relates to sediments.

Chemicals with the potential to cause adverse effects to humans are identified during the hazard identification step. This includes an understanding of nature and extent of contamination, fate and transport of chemicals, and whether chemicals exceed applicable human health screening levels. In evaluating sediments, considerations in this step include characteristics of the waterbody (e.g., on-site drainage channel, river); whether the sediments are submerged for a full year or only a portion of the year; migration of chemicals in the water bodies; and available and applicable screening levels (i.e., soil, sediment, both).

An exposure assessment evaluates receptors that may be exposed to the impacted sediment. Exposure factors and assumptions may differ from default exposure assumptions because activities are specific to individual sites. Exposure assumptions rely on understanding the activities of the local population. The presentation will discuss specific examples of these differences including assumptions associated with different types of recreational activities and exposure point concentration determinations for direct contact or consumption of fish.

In the toxicity assessment step, the presentation will review the relationship between the potential extent of exposure and toxicological effects of the exposure. It is important to understand the form and bioavailability of the chemical within the sediment, as toxicity will vary depending on these factors.

Risk characterization incorporates the information from the preceding three steps and provides an understanding of the order of magnitude of potential adverse health effects from exposure to impacted sediments. The results can be considered in context of regional or site-specific background concentrations and sensitivity analyses can be performed to help guide remedial decisions.

**6.02A.T-06 Weight of Evidence Identifying Lower Beaverdam Creek as a Current Source of Bioavailable PCBs in the Anacostia River, USA**

***Alfred Pinkney***[1]*, Elgin Perry, Lance T. Yonkos*[2]*, Dev Murali*[3]*, Nathalie Lombard*[5]*, Mandar Bokare*[4] *and Upal Ghosh*[4]*, (1) U.S. Fish and Wildlife Service, (2) University of Maryland, (3) Department of Energy and Environment, Canada, (4)University of Maryland, Baltimore County*

The District of Columbia Department of Energy and Environment (DOEE) has been investigating contamination in the Anacostia River, Kingman Lake, and Washington Channel in Washington, DC and Maryland, USA over the past ten years. Elevated concentrations of polychlorinated biphenyls (PCBs) and organochlorine (OC) pesticides in tidal river sediments pose a risk to aquatic biota and human health. DOEE has also funded and coordinated studies of Anacostia tributaries to determine sources of ongoing contamination. A U.S. Geological Survey study found that Lower Beaverdam Creek (LBC), a Maryland tributary that provides 15 percent of the flow to the tidal river contributed about 75 percent of total PCB loading in the form of suspended sediments. Freely dissolved PCBs, based on deployments of passive samplers, detected highly elevated concentrations in two LBC locations near suspected source areas and contributed to a high dissolved PCB load to the Anacostia River. Similarly, high PCB concentrations were observed in caged *Elliptio* *complanata* freshwater mussels deployed at LBC. Most recently, stationary forage fish (mummichogs, *Fundulus heteroclitus*, and banded killifish, *F. diaphanus*) sampled at these LBC locations had mean whole body total PCB concentrations of 313 to 1110 parts per billion (ppb). In contrast, mean total PCB concentrations in forage fish from the Northwest and Northeast Branches, which together contribute about 75 percent of the tributary flow to the tidal river, ranged from 47.9 to 76.5 ppb. Forage fish, passive samplers, and mussels from LBC were enriched in lower chlorinated PCB homologs, indicative of current loading. Findings from a linked food web/mass balance model indicates that drastic reductions in LBC PCB loading would have a major impact in reducing concentrations in forage and game fish. Ongoing investigations have identified a soil hot spot (total PCBs greater than 50 ppm) at a a recycling facility (one of the source areas) resulting in source removals. We developed a linked food web/mass balance model which demonstrated the importance of a mechanistic approach. It showed that source control in the tributaries coupled with sediment cleanup actions would provide the maximum recovery in the tidal Anacostia River mainstem. The post-remedial monitoring of contaminants in forage fish, freshwater mussels, and passive samplers will provide supporting data on the effectiveness of source control and remedial actions.

**6.02A.T-07 Equivalence Testing and the Reverse Null Hypothesis: Assessing Progress Towards Cleanup Levels at Portland Harbor**

***Kyle Edward Vickstrom*[1]**, *Jason Silvertooth[1], John Kern[2], Sean A. Sheldrake[1] and Madi Novak[3], (1) CDM Smith, (2) Kern Statistical Services, (3) U.S. Environmental Protection Agency*

The Portland Harbor Superfund Site (site) is an approximately 10-mile reach of the lower Willamette River in Portland, Oregon that has contaminated sediment, riverbank soil, surface water, groundwater, and biota for which a Record of Decision (ROD) was signed in 2017. Focused contaminants of concern (COCs) include polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), dichlorodiphenyltrichloroethane and its derivatives (DDx), and polychlorinated dibenzo-p-dioxins and furans (dioxins/furans). The ROD established sediment cleanup levels based on risk values or background concentrations in the reference area (Upriver Reach), but not a statistical approach for determining when background-based cleanup levels are attained. Equivalence testing using the reverse null hypothesis is a robust method for comparing two datasets and can determine with statistical reliability whether the site and reference area are equivalent within scientifically meaningful margins of error. For this study, an equivalence factor was established with self-equivalence simulations using a modeled sample distribution of Upriver Reach data. Different sample sizes were evaluated to determine the relationship between sample size and the equivalence factor and to select an appropriate equivalence factor for the 2018 baseline sampling data (424 site and 30 reference area surface sediment samples). These data were evaluated for equivalence for PCBs, PAHs, DDx, and dioxins/furans by calculating the ratio of geometric means and its 95% upper confidence limit between the site and Upriver Reach and comparing the results to the simulated equivalence factor. Equivalence testing with the 2018 unbiased baseline data show that focused COC sediment concentrations at the site are not statistically equivalent with the Upriver Reach at their simulated equivalence factor. Collection of additional rounds of unbiased sediment data will allow statistically robust assessment of temporal trends and future evaluations of equivalence during and after remediation. Furthermore, additional data will refine the Upriver Reach self-equivalence evaluation and allow for determination of a robust equivalence factor. This study demonstrated statistically meaningful methods for evaluating equivalence between datasets that can be used to assess progress towards achieving cleanup goals and help determine whether these background-based goals may require adjustment in future five-year reviews.

**6.02A.T-08 Poster Highlights 1 of 2: Contaminated Sediment Toxicity, Risk Assessment and Management, Remediation, Restoration, Climate Change Resiliency**

***Bonnie Brooks***, *Washington State Department of Ecology*

In this poster highlights time slot, the authors of some of the excellent posters submitted to the session will provide a overview slide presentation to highlight the major findings in their poster presentation.

## 6.02B Contaminated Sediment Toxicity, Risk Assessment and Management, Remediation, Restoration, Climate Change Resiliency

### 6.02B.T-01 Drain the Pond, Fix the Pond, Make Happy Turtles: Sediment Restoration at the Columbia Slough in Oregon

*Phil Wiescher and **Erik Naylor**, Maul Foster & Alongi, Inc.*

Since 1994, a metal-recycling operations were conducted adjacent to a shallow pond that is hydraulically connected to the Columbia Slough, a large water control and habitat system in Portland, Oregon. In accordance with a Record of Decision issued by the Oregon DEQ, a remedial action to address the presence of polychlorinated biphenyls (PCBs), metals, and polycyclic aromatic hydrocarbons in contaminated soil and sediment was identified.

MFA prepared and implemented the remedial design which included excavation and off-site disposal of sediment and soils with relatively higher concentrations of contaminants, placement of a thin layer of clean sand blended with activated carbon in areas with moderate PCB concentrations, and placement of a thin layer of clean sand to enhance natural recovery in peripheral areas. Due to the shallow water depth, which precluded the use of marine equipment, the pond was dewatered to allow construction of an access road for land-based equipment. The road was removed and the pond bottom restored following placement of clean and amended sand. MFA acted as the general contractor and implementation occurred in summer 2021.

The project also included enhancements over and above the requirements of the remedy to address state natural resource damages and improve native turtle habitat. MFA enhanced the pond bank by grading and landscaping it to promote turtle nesting and create a migration corridor to the Whitaker Slough. Turtle habitat was further improved by installing basking structures, constructed with salvaged logs and root wads from site clearing, to limit access by terrestrial predators.

As part of the design plan, MFA conducted pre-remedial pore water sampling to quantify the presence of PCBs that are biologically available for uptake by ecological receptors. MFA will use the sample results as a baseline for comparison to post-remedial pore water values to confirm that the selected cleanup actions continue to protect human health and the environment.

The site is located in the Whitaker Ponds Nature Park and is owned by multiple public and private parties. In addition, the Columbia Slough Watershed Council is an important stakeholder whose office is located in the nature park. To convey the importance and benefits of the site cleanup, MFA and MMNW engaged the Columbia Slough Watershed Council. MFA continued public outreach throughout the remedial design and implementation.

### 6.02B.T-02 The impact of biotransformation by benthic invertebrates for fate and bioaccumulation of sediment-associated hydrophobic organic contaminants

***Henriette Selck**, Roskilde University, Denmark*

In general, regulatory frameworks assess risk of hydrophobic organic chemicals (HOCs) based on their persistence, bioaccumulation and toxicity using water-exposure setups and microbial degradation. However, this may misjudge the risk of HOCs, such as fragrance materials (FMs) and cyclic volatile methyl siloxanes (cVMS), due to their accumulation in the sediment compartment. We assessed the impact of dietary uptake (i.e., via sediment ingestion) and benthic invertebrate biotransformation for fate and bioaccumulation of HOCs in several studies with a freshwater oligochaete (*Tubifex tubifex*) and a marine polychaete (*Capitella teleta*). Overall, sediment–associated HOCs did not adversely affect the benthic invertebrates. Uptake, depuration and biotransformation was compound- and species specific. BSAF was low due to biotransformation and fast elimination of parent and metabolites. Worm presence reduced sediment HOCs (up to > 95% ), especially in the estuarine system inhabited by *C. teleta*, to levels exceeding microbial degradation alone. Our results highlight the importance of benthic invertebrate biotransformation in reducing uncertainty in B and P assessment of HOCs.

**6.02B.T-04 Moving Towards a Chemical Activity-Based Risk Assessment of Sediments**

***Sebastian Abel****, Sophie Steigerwald, Gastón Alurralde, Ann-Kristin Eriksson-Wiklund, Elena Gorokhova and Anna Sobek, Stockholm University, Sweden*

Risk assessment of sediment-associated hydrophobic organic contaminants (HOC) is commonly based on measured concentrations that are normalized to the sediment organic carbon or dry weight. However, this approach neglects other factors influencing the bioavailability of a given contaminant. Instead, risk assessment based on the HOC chemical activity, which is closely related to the freely dissolved concentration ($C_{free}$), would provide a more relevant assessment. Furthermore, the chemical activity can simplify the prediction of mixture toxicity, bioaccumulation, and biomagnification. To improve our understanding of the relationships between chemical activity and biological effects, we need bioassay systems that are designed to apply chemical activity as a dose metric. We are developing such sediment-water test systems for use in assays with benthic invertebrates. Instead of conventionally spiking artificial sediment to the target concentration, it is loaded through a saturated solution of the HOCs of interest. A sealed polyethylene-bag permeable for dissolved HOCs is filled with a suspension of crystalline HOCs and submerged in the loading vessel; the bag serves as a reservoir for the test substance to avoid depletion of the saturated water. Once fully loaded (saturated), the target chemical activity of the sediment can be adjusted by mixing it with clean sediment. Unlike the conventional spiking, this approach yields comparable $C_{free}$ and thus stable HOC bioavailability regardless of the sediment characteristics (e.g. quantity and quality of organic carbon). In a pilot study, we used this approach to load peat with 4 different PAHs. The peat was freely suspended in the saturated, aqueous loading solution and regular subsamples were taken over the course of 6.5 weeks. Following an accelerated solvent extraction, the PAHs were analyzed in the peat samples using GC-MS. The results indicate a 2.5 to 4.5 week equilibration time until the peat reached saturation. In a follow-up test, the successfully loaded peat will be diluted with clean peat and used for the exposure of the benthic invertebrate *Hyalella azteca*, and PAH concentrations in the sediment and the animals will be measured and used to validate the presented approach for chemical activity-based sediment bioassays, mixture toxicity, and bioaccumulation estimates.

**6.02B.T-05 Developing Hydrocarbon PRGs Using Passive Sampling, Porewater, and Bulk Sediment**

*Charles Nace[1], **Dan Cooke**[2], David Mount[1], Robert M. Burgess[1] and Lawrence Burkhard[1], (1) U.S. Environmental Protection Agency, (2) CDM Smith*

**Background/Objectives:** At a large Superfund sediment site in the northeastern United States with significant petrochemical contamination, the US Environmental Protection Agency (EPA) used the sediment porewater concentrations of 34 polycyclic aromatic hydrocarbons (PAH (34)) to derive preliminary remediation goals (PRGs) in accordance with EPA's 2017 guidance document, "*Developing Sediment Remediation Goals at Superfund Sites Based on Pore Water for the Protection of Benthic Organisms from Direct Toxicity to Non-ionic Organic Contaminants*". The use of sediment porewater, based on passive sampling to incorporate equilibrium partitioning of contaminants between bulk sediment and the bioavailable fraction, represents the state-of-the-science. Sediments with porewater PAH concentrations exceeding EPA's equilibrium partitioning sediment benchmarks (ESB) were consistently toxic, but not all toxic sediments had high PAH concentrations in porewater. This inconsistency led to the development of an alternative approach to deriving PRGs.

**Approach/Activities:** While performing a baseline ecological risk assessment (BERA), the Responsible Parties collected a series of 35 site sediment samples for evaluation using the sediment quality triad approach (SQT; benthic community, sediment chemistry, and toxicity testing). While sediments with high porewater PAH (34) concentrations were toxic, dilution-based PRGs calculated from the observed $K_{OC}$ values led to unrealistically low bulk phase PRGs. Sediment toxicity correlated strongly with several other classes of hydrocarbon compounds (e.g., C9-C40 total petroleum hydrocarbons, C10-C28 diesel range organics, C19-C36 alkyl hydrocarbons) suggesting that several samples had toxicity attributable to alkyl hydrocarbons instead of or in addition to toxicity from PAHs. Exposure response relationships to multiple indices of hydrocarbon contamination yielded PRG values that effectively parsed toxic and non-toxic samples.

**Results/Lessons Learned:** EPA's 2017 porewater remediation guidance states that the method will not be suitable for all sites, and in such cases, the alternate approach used for this site may allow defensible derivation of PRGs. Consequently, EPA derived PRGs for multiple hydrocarbon classes and found that combining both PAH (34) and the C19-C36 fractions yielded PRGs that addressed toxicity at all 35 SQT locations. This abstract does not necessarily reflect US EPA policy

### 6.02B.T-06 Determining the Leaching Potential of Dredged Sediments From the Bunker Hill Superfund Site

***Erik Naylor**, Maul Foster & Alongi, Inc.*

Sediments in the Lower Basin of the Coeur d'Alene River in the Bunker Hill Superfund Site are impacted by metals-contaminated tailings and mine wastes that have entered Upper Basin tributaries of the Coeur d'Alene River and have been conveyed to downstream areas. The process of downstream fluvial transport of contaminated sediments and deposition of the contaminated sediments on floodplains, stream banks, and similar areas in the Lower Basin is ongoing. Once impacted sediments are deposited, stream bed transport and bank erosion may redistribute them throughout the Lower Basin. The contaminated sediment and streambeds need to be remediated to prevent ongoing contamination. The Dudley Reach is the segment of the Lower Coeur d'Alene River from river mile 154 to 160, as defined in the Record of Decision. An investigation focused on 0.9 miles of the upper Dudley Reach where an initial pilot project will eventually be sited and designed for sediment remediation. Riverbed sediments were evaluated to assess the nature and extent of impacted materials, geotechnical properties of sediments, and leachability of cadmium, lead, and zinc in sediments. Additionally, riverbank sediments were evaluated to assess metals concentrations and erosion rates along the project area. Two U.S. Environmental Protection Agency Leaching Environmental Assessment Framework (LEAF) methods were used to determine the leaching potential of dredged sediments after placement in a waste consolidation area. The LEAF methods selected simulated a groundwater flowthrough and rainwater infiltration scenario (LEAF 1314), and an intermittent flooding scenario (LEAF 1315). The leachates generated by the LEAF methods were analyzed for dissolved metals, hardness, and general field parameters. In Addition to LEAF analysis, the sediment samples were also analyzed for grain size and total metals content. Metals were found to leach at very different rates using both LEAF methods and there was not a clear correlation to total metals content of the sediment. The data indicates that there is potential for leaching of cadmium, lead, and zinc from the sediments that would result in concentrations higher than ambient water quality criteria. The results of the study will be interpreted for use in the next phase of the remedial design.

### 6.02B.T-07 TIGSED: A Modeling Approach for Assessing Source Control Sufficiency

***Nicholas Rose**, Philip Spadaro, Jason Dittman and Bei Chu, TIG Environmental*

Source control sufficiency assessments are becoming an important part of sediment remediation, particularly in the Pacific Northwest where EPA has begun requiring them as part of the design process for both the Portland Harbor Superfund Site and the Lower Duwamish Waterway. These assessments are necessary to ensure that expensive in-water remediation is not re-contaminated in the near or long term from existing upland contributions. Large river systems are complex with many potential in-water and upland sources that may contribute to contamination in the sediment. Thus, a source control sufficiency assessment must account for all these contributions to accurately assess the potential for recontamination and, if needed, identify mechanisms to prevent recontamination. This presentation will discuss TIGSED, a modeling approach based on the SEDCAM model that can be used as a line of evidence to determine if sources are sufficiently controlled and, if not, help identify specific sources to control. TIGSED was developed to handle the complexity of assessing contaminant contributions to sediments in large river systems while being flexible enough to adapt to the specific conceptual model of a given system or area. The model relies on site-specific information and can be conducted at various scales depending on the system under consideration and needs of the analysis.

**6.02B.T-03 Characterizing Site-Specific Human Health and Ecological Risks from Potential Exposure to Constituents of Potential Concern in Fish and Crawfish Tissue from a Southern Louisiana River**

***Andrew Miano**, Greg Murphy and Thomas Biksey, EHS Support LLC*

Characterizing site-specific human health and ecological risks from potential exposure to constituents of potential concern (COPCs) in fish tissue from large river systems can be fraught with challenges. We conducted a field investigation to characterize human health and ecological risk from potential exposure to inorganic and organic COPCs in fish and crawfish tissue from a large, tidal river system in southern Louisiana. Uncertainty within the risk assessment stemmed from the influence of site-specific and regional physical and physio-chemical habitat conditions on the relative abundance of fish and crawfish, the influence of regional background conditions on COPC concentrations in fish tissue, and agency concerns regarding the use of biota-sediment accumulation factors (BSAFs) to estimate concentrations of COPCs in fish and crawfish tissue. We leveraged site-specific habitat reconnaissance, existing information, and interviews with local Fish Consumption Advisory Committee members to design our investigation. The results of our field investigation will be used to characterize site-specific risks from potential exposure to inorganic and organic COPCs in fish and crawfish tissue. Results of this investigation can be used to inform other investigations on complex river systems where similar uncertainties may be encountered.

**6.02B.T-08 Poster Highlights 2 of 2: Contaminated Sediment Toxicity, Risk Assessment and Management, Remediation, Restoration, Climate Change Resiliency**

***Bonnie Brooks**, Washington State Department of Ecology*

In this poster highlights time slot, the authors of some of the excellent posters submitted to the session will provide a overview slide presentation to highlight the major findings in their poster presentation.

**6.03 Conventional and Unconventional Oil in the Environment: Characterization, Environmental Pathways, Toxicity and Treatment**

**6.03.T-03 Photocatalytic Degradation of Polycyclic Aromatic Hydrocarbons in Water by 3D printed TiO2 Composites**

***Andrew D. McQueen**[1], Mark Ballentine[2], Lauren May[2], Charles Laber[2], Michael Bortner[3], Arit Das[3] and Alan Kennedy[2], (1) U.S. Army Engineer Research and Development Center, (2) U.S. Army Corps of Engineers, (3) Virginia Polytechnic Institute and State University*

Recent progress in developing composites embedded with photocatalysts indicates application for treatment of organic contaminants in aquatic systems. However, significant gaps remain in developing effective photocatalyst polymer composites for use as customizable, deployable, and retrievable structures for mitigating environmental contamination. The goal of this study was to generate and evaluate the performance of 3D printed $TiO_2$ composites for degrading polycyclic aromatic hydrocarbons (PAHs) in waters affected by contaminated sediment. Photocatalytic structures were fabricated using polylactic acid (PLA) compounded with $TiO_2$ nanoparticles as filament feedstock and printed using a benchtop 3D printer. Photocatalysis and photolysis experiments were conducted in controlled environmental chambers under full spectrum light ($\lambda$ 280-750 nm). 3D printed PLA- $TiO_2$ disks increased the degradation kinetics (compared to photolysis) of a complex mixture of 4- to 5-ring PAHs achieving nondetectable concentrations within hours to days. The PAH removal rate was relatively rapid, with 3D printed PLA-$TiO_2$ treatments achieving degradation half-lives within ~6 to ~24 h. After 48 h of treatment, both photolysis and photocatalysis eliminated toxicity to *Ceriodaphnia dubia*. These data indicate the potential application of 3D printable photocatalytic polymers to immobilize $TiO_2$ for targeted mitigation of problematic organic constituents in water and highlight the benefits of additive manufacturing to rapidly prototype and optimize innovative structures.

**6.03.T-04 AEOs Fractionation Reveals that Acute Toxicity is Mainly Due to Naphthenic Acids**
***Juan Manuel Gutierrez-Villagomez***[1]*, Juan Vázquez Martínez*[2]*, Wo Su Zhang*[3]*, Enrique Ramírez Chávez*[4]*, Jorge Molina Torres*[4] *and Vance L. Trudeau*[5]*, (1) Institut National de la Recherche Scientifique (INRS), Canada, (2)Instituto Tecnologico Superior de Irapuato (ITESI), Mexico, (3)University of Ottawa, Canada, (4) Cinvestav Unidad Irapuato, Mexico, (5) University of Ottawa, Canada*
The oil industry is economically important worldwide; however, there are still concerns regarding the exposure to oil and its by-products. Naphthenic acids (NAs) are oil-derived complex mixtures of carboxylic acids that can be released to the environment after oil spills. High concentrations of NAs are found in tailings ponds in the oil sands mining region of Alberta, Canada. Open column chromatography was used to fractionate a commercial naphthenic acid (NA) mixture and an acid extractable organic (AEO) mixture derived from an oil sands process-affected water (OSPW) to identify the components responsible of the toxicity of these mixtures. The mixtures and the fractions were analyzed as PFBBr derivatives by GC-EIMS specifically for NAs. The lethal and sublethal toxicity of the fractions was later assessed using *Silurana (Xenopus) tropicalis* embryos. The commercial extract and its polar fraction decreased survival rate, total length (TL), tail length (TaL), snout-vent length (SVL), and interorbital distance (IOD) ($p < 0.05$) of *S. tropicalis* embryos. The AEO extract reduced survival rate, TL, TaL, SVL, and IOD ($p < 0.05$). The exposure to the more polar fraction did not decrease the survival up to 48 mg/L ($p > 0.05$), but it decreased the TL, TaL, SVL, and IOD of *S. tropicalis* at 48 mg/L ($p < 0.05$). However, the polar fraction was significantly less toxic than the AEOs ($p < 0.05$). The exposure to the less polar fraction did not have any significant effect on *S. tropicalis* ($p > 0.05$). Our data indicates that the acute toxicity of NA mixtures is mainly due to the presence of NAs. Furthermore, the AEOs toxicity is mostly due to its polar components and the fractionation reduced the general toxicity.

**6.03.T-05 Lethal and Sub-Lethal Effects of the Photo-Enhanced Toxicity of Diluted Bitumen, Crude Oil and UV Radiation on Hyalella azteca from the IISD-ELA Freshwater Oil Spill Remediation Study (FOReSt).**
***Sonya Marie Michaleski***[1]*, Adrienne Bartlett*[2]*, Lauren Timlick*[3]*, Mace Barron*[4]*, Lisa Peters*[3]*, Kenneth Jeffries*[5] *and Vince Palace*[3]*, (1) University of Manitoba, Canada, (2 )Environment and Climate Change Canada, (3) IISD Experimental Lakes Area (IISD-ELA), Canada, (4) U.S. Environmental Protection Agency, (5) University of Manitoba, Canada*
Canada is one of the top oil producers globally with approximately 10% of the world's oil reserves. Crude oils and diluted bitumen (bitumen diluted with natural gas condensates) are the major products of the Canadian oil sands region. Potential environmental impacts of oil spills are a concern for the public and the oil industry. Pipelines are statistically the safest transportation method for oil in Canada. The rate of crude oil and diluted bitumen (dilbit) spills has declined over the past decade, however, many pipelines and proposed pipelines cross freshwater, and more knowledge is required about how oil behaves in these systems. Previous studies have evaluated the toxicity of crude oils and dilbit, but many overlook the potential for photo-enhanced toxicity of oil constituents, specifically polycyclic aromatic compounds. Photo-enhanced toxicity is a synergistic interaction between the toxicity of a contaminant and UV radiation. This phenomenon can increase the toxicity of a contaminate anywhere between 2-1000 times.

The Freshwater Oil Spill Remediation Study (FOReSt) at the IISD-Experimental Lakes Area was designed to study the effects of oil spills in a shoreline environment and compare the efficiency of non-invasive methods to remediate freshwater shorelines after oil spills. These secondary remediation measures included engineered floating wetlands, nutrient enhanced monitored natural recovery, and COREXIT EC9580A shoreline cleaner. This study was conducted with cold lake blend dilbit in 2019 and conventional heavy crude oil in 2021.

To examine the photo-enhanced toxicity of these oils, a separate study was conducted where *Hyalella azteca* were exposed to water accommodated fractions (WAFs) of dilbit (2019) and crude oil (2021) from oil weathering experiments and shoreline enclosures, both a part of the larger FOReSt study. The test was then duplicated to expose individuals to low (10%) and high (90%) UV exposures. Mortality was documented throughout a 5-day exposure, and photographs of living individuals were taken at the end. Using the photos, growth was measured, and a deformity analysis was conducted to identify and categorize sub-lethal impacts of remediation measures and photo-enhanced toxicity. Overall, both dilbit and crude oil exhibited photo-enhanced toxicity to *Hyalella azteca* as indicated by increased mortality and deformities, and decreased growth.

**6.03.T-06 Using Biological Responses to Monitor Freshwater Post-Spill Conditions over 3 years in Blacktail Creek, North Dakota, USA**

***Aïda Farag***, *David Harper, Isabelle Cozzarelli, Doug Kent, Adam Mumford, Denise Akob, Travis Schaeffer and Luke R. Iwanowicz, U.S. Geological Survey*

A pipeline carrying unconventional oil and gas (OG) wastewater spilled approximately 11 million liters of wastewater into Blacktail Creek, North Dakota, USA. Flow of the mixed stream water and wastewater down the channel resulted in storage of contaminants in the hyporheic zone and along the banks, providing a long-term source of wastewater constituents to the stream. A multi-level investigation was used to assess the potential effects of oil and brine spills on aquatic life. In this study, we used a combination of experiments using a native fish species, Fathead Minnow (*Pimephales promelas*), field sampling of the microbial community structure, and measures of estrogenicity. The fish investigation included in situ experiments and experiments with collected site water. Estrogenicity was measured in collected site water samples and microbial community analyses were conducted on collected sediments. During the initial post-spill investigation, February 2015, performing in situ fish bioassays was impossible because of ice conditions. However, microbial community (e.g., the presence of members of the Halomonadaceae, a family that is indicative of elevated salinity) and estrogenicity differences were compared to reference sites and point to early biological effects of the spill. We noted water column effects on in situ fish survival 6 months post-spill during June 2015. At that time, total dissolved ammonium (sum of ammonium and ammonia, TAN) was 4.41 mg/L with an associated $NH_3$ of 1.09 mg/L, a concentration greater than the water quality criteria established to protect aquatic life. Biological measurements in the sediment defined early and long-lasting effects of the spill on aquatic resources. The microbial community structure was affected during all sampling events. Therefore, sediment may act as a sink for constituents spilled and as such provide an indication of continued and cumulative effects post-spill. However, lack of later water column effects may reflect pulse hyporheic flow of ammonia from shallow ground water. By understanding effects at these various levels of biological organization, natural resource managers can interpret how a course of action, especially for remediation/restoration, might affect a larger group of organisms in the system.

**6.03.T-07 Characterizing the Geochemical Evolution and Aquatic Toxicity of a Diluted Bitumen Spilled Within Shallow Groundwater Systems**

***Scott Leslie Hepditch*[1]**, *Tuan Anh To[1], Ève Larocque[1], Juan Manuel Gutierrez-Villagomez[1], Jason M. E. Ahad[2], Richard Martel[1] and Valerie S. Langlois[1], (1)Institut National de la Recherche Scientifique (INRS), Canada, (2) Natural Resources Canada, Canada*

Pipelines efficiently transport diluted bitumen (dilbit), which contains ~70% bitumen blended with lighter hydrocarbon fractions to reduce viscosity and facilitate flow. Increased interest in Canadian dilbit and previous spill events have emphasised the need to study dilbit fate and behaviour, yet investigations into the evolution of subsurface plumes within shallow groundwater systems are lacking. Thus, it is currently unknown if the geochemical characteristics and resultant toxicity of spilled dilbit over time differ from those of more well studied conventional crudes. To this end, separate controlled spill experiments were conducted for several months in large (1 × 0.6 m) unsaturated soil columns with Cold Lake Blend dilbit and a comparative pooled sample of conventional heavy crude with similar physical and chemical properties. Watering regimes of 22 mm per week were used, with leachate samples routinely collected. Total organic and inorganic carbon increased over time and concentrations of BTEX (benzene, toluene, ethylbenzene and xylenes) were two-fold greater in the dilbit column compared to the conventional crude column 14 days post-spill. Concentrations of polycyclic aromatic hydrocarbons (PAHs) were low (parts per trillion, ng $L^{-1}$) although showed a gradual increase with time. Acid extractable organics (AEOs) containing naphthenic acids were also generally low (< 2 mg $L\text{-}^{1}$) and mainly dominated by naturally occurring fatty acids. Fathead minnow embryotoxicity analyses with leachate water identified the severe effects upon fish survival 34-days post-spill, with a significantly greater prevalence of malformations and expression of *cyp1a*, but with no differences observed between oil types. Toxic effects did not correlate with measured PAHs, BTEX, metals, or AEOs, highlighting the need to further characterize the understudied spectrum of petroleum constituents that may pose risks to aquatic systems. This study provides a temporal profile of the geochemical behaviour and toxicological impact of dilbit in the shallow unsaturated zone following a spill.

**6.03.T-08 Assessing the Efficacy of a Remediation Product for Degrading Polycyclic Aromatic Compounds (PACs) After Spills of Conventional Heavy Crude Oil into Freshwater**

***Blake T. Cooney**[1], Lisa Peters[2], Nicholas Blandford[1], Gregg Tomy[1], Mark L. Hanson[1], Robert Menegotto[3], Janet Angel[4], Michael Welch[4] and Vince Palace[2], (1) University of Manitoba, Canada, (2) IISD Experimental Lakes Area (IISD-ELA), Canada, (3) Mantech, Inc, Canada, (4) Jacor, LLC*

Conventional heavy crude oil (CHV) is a common oil product transported through pipelines to markets for shipping or refinement. In the scenario of an aquatic oil spill, remediation of affected environments is a crucial consideration. EcoBioClean ® (EBC) is a proprietary lyophilized product consisting of nutrients, dispersants, and bacterial inoculant applied to oil spill affected areas as a passive remediation technique. The fate of polycyclic aromatic compounds (PACs) and nutrients (C, N, & P) are important factors during remediation. We assessed the efficacy of EBC for remediating PAC concentrations in model freshwater systems affected by oil spills. Twelve glass beakers, containing 1 litre of lake water and 100g of wet sediment, collected from Lake 260 at the IISD Experimental Lakes Area, were treated in triplicate with either a) weathered CHV and EBC, b) weathered CHV only, c) EBC only, d) untreated control systems. Water sampled on days 0,1,4,9,16, and 32 was analyzed immediately for photoelectric chemical oxygen demand (peCOD), while samples for parent, as well as alkylated PACs (aPACs) were extracted the same day for future analysis using GC-MS/MS. Bulk sediment for PAC analysis, as well as water for nutrient chemistry, was sampled on days 0 and 32. peCOD measures detected significant differences in the dissolved fraction at $T_1$ (control=17.76 ± 4.61 mg/L, EBC=223.59 ± 8.71 mg/L, CHV=24.09 ± 2.05 mg/L, EBC+CHV=168.65 ± 21.34 mg/L). Over 90% of observed PACs were alkylated. EBC treated oil resulted in lower C2 naphthalene concentrations by day 32 (EBC+CHV=76±23 ng/L, CHV=556±129 ng/L) as well as lower C3 napthalene concentrations (EBC+CHV=173±15 ng/L, CHV=668±33 ng/L). Ongoing analysis is being undertaken on sediment PAC concentrations, and additional aPACs to support these initial results, which indicate an increase in degradation of PACs and dissolved organic carbon in EBC+CHV treatments compared to CHV treatments.

**6.03.P Conventional and Unconventional Oil in the Environment: Characterization, Environmental Pathways, Toxicity and Treatment**

**6.03.P-We184 Characterising the geochemical evolution of a diluted bitumen spill within the saturated zone**

***Scott Leslie Hepditch**[1], Jason M.E. Ahad[2], Thomas Robert[1], Tuan Anh To[1], Ève Larocque[1], Juan Manuel Gutierrez-Villagomez[1], Gaëlle Triffault-Bouchet[3], Richard Martel[1] and Valerie S. Langlois[1], (1) Institut National de la Recherche Scientifique (INRS), Canada, (2) Natural Resources Canada, (3) Ministère de l'Environnement et de la Lutte Contre les Changements Climatiques (MELCC), Canada*

Pipelines efficiently transport diluted bitumen (dilbit), which contains ~70% bitumen blended with lighter hydrocarbon fractions to reduce viscosity and facilitate flow. Increased interest in Canadian dilbit and previous spill events have emphasised the need to study dilbit fate and behaviour into the environment, yet investigations into the evolution of subsurface plumes within shallow groundwater systems are non-existent. To address this research gap, we carried out a controlled spill of 15 kg of dilbit (Cold Lake Blend) buried within a large sand-tank (12 $m^3$) containing 11,000 kg of sand receiving a 1,000 L unidirectional-flow of water per day to mimic a below-ground spill of dilbit within the saturated zone. A suite of geochemical parameters were analyzed over a period of 3.5 months from soil cores and water samples collected from a series of piezometers of varying distances and depths from the dilbit source zone. Petroleum contamination was greatest in the days shortly following the spill event, with total organic carbon and volatile organic compounds decreasing in concentration over time. Greater concentrations of dilbit were observed in the upper portions of the sand-tank, reflected by depleted $\delta^{13}C$ values, and increased polycyclic aromatic hydrocarbon and volatile organic compound concentrations. Dissolved oxygen concentrations were depleted within the upper portions of the sand-tank, likely suggesting oxygen dependent processes, such as microbial degradation of the dilbit. Water at the outflow of the system, representative of groundwater discharging in a surface water body, was collected for weekly 7-day EPA embryologic teratogenicity and monthly 7-day EPA larval toxicity assays with fathead minnows (*Pimephales promelas*). No effects were observed for embryologically exposed fish, however, growth was reduced for larval fish exposed to water 30 days post-spill, but not 60 days post-spill. Compared to previous studies on the aquatic toxicity of dilbit, PAH concentrations remained relatively low and consistent throughout all the exposures (below 12 $\mu g\ L^{-1}$), while BTEX concentrations were high, reaching 1.7 $mg\ L^{-1}$ and lowering to 0.2 $mg\ L^{-1}$ immediately following the spill and 70-days post-spill, respectively. This experiment provides a temporal profile of an underground dilbit spill over time and identifies the risk that such spills pose on associated freshwater systems.

### 6.03.P-We185 Connecting gut microbiome changes with disease in fish exposed to oil

***Giovanna Monticelli***[1], *Jason Tyler Magnuson*[1], *Daniel Schlenk*[2], *Joseph H. Bisesi Jr.*[3] *and Daniela Maria Pampanin*[1], *(1) University of Stavanger, Norway, (2) University of California, Riverside, (3) University of Florida*

The gut microbiome is considered a key factor in the health status of organisms. Exposure to contaminants, such as polycyclic aromatic hydrocarbons (PAHs), could significantly affect the community structure, and gene expression of the gut microbiome. The aim of our study was to assess the potential shift in the gut bacterial community composition and activity, and its role on fish gut metabolism following an oil exposure. Juvenile Atlantic cod (*Gadus morhua*) were exposed to dispersed crude oil (DCO) (0.05 ppm for 1, 3, 7, and 28 days) to simulate the potential contamination related to produced water discharge at sea. Metagenomic analyses (16S rRNA) were performed to characterize the species composition, richness and diversity of the gut microbial community. In addition, RNA-sequencing was also conducted to provide gene expression information within the gut microbiome and its host. An Illumina MiSeq platform was used for metabarcoding (V4-V5 region) and Illumina NextSeq for mRNA sequencing. The loss of microbiota richness, highlighted by the decrease in the alpha diversity indices in the DCO exposed groups, may compromise fish behaviour and ability to respond to environmental changes. The gut microbiome taxonomy data revealed that: i) the most abundant orders were Vibrionales, Mycoplasmatales, Actinobacteridae and Alteromonadales; ii) the most abundant genera were *Photobacterium*, *Aliivibrio* and *Mycoplasma* and a change of those genera was observed over time. In the metatranscriptome, five significant differentially expressed genes (DEGs) were identified, which were involved in nucleotide-binding of sugar metabolism enzyme, peptidase and glutathione S-transferase. DEGs from the faecal fish transcriptome were analyzed through the predictive pathway analysis software Ingenuity Pathway Analysis (IPA). Gastrointestinal disease was among the top predicted diseases based on function pathways after 1 day of exposure. The top predicted canonical pathway after 3, 7, and 28 days of exposure was EIF2 signaling, which plays a key role in global translation initiation and protein synthesis. In addition to gastrointestinal disease, RNA damage, and altered protein synthesis after 3, 7, and 28 days of exposure was predicted based on transcriptomic signatures from the fish. This study shows that DCO may impair the fish gut microbiome and highlights key gaps to efficiently connect gut microbiome changes and fish health conditions.

**6.03.P-We186 Assessing the Effects of Ultraviolet Light on the Toxicity of Individual Aromatic Compounds and Crude Oils to Two Life Stages of Atlantic Cod (Gadus morhua)**

***Danielle A. Philibert***[1]*, Thomas Parkerton*[2] *and Benjamin Patrick de Jourdan*[1]*, (1) Huntsman Marine Science Center, Canada, (2) EnviSci Consulting*

Ultraviolet (UV) light plays a significant role in the fate and biological effects of an oil spill through two main processes, photo-modification and photo-sensitization. It is estimated that nearly 10% of the oil in the *Deepwater Horizon* oil spill was transformed into oxygenated compounds through photo-modification. Photo-sensitization occurs when an organism has taken up a photodynamic compound and then is exposed to sufficient light, resulting in greater toxicity. The objective of this study is to better understand the toxicological significance of photo-modification and photo-sensitization for species of cultural and commercial significance to provide data inputs needed for oil spill models. Atlantic cod (*Gadus morhua)* embryos and larvae spend the majority of their time at the surface of the water column which make them a good candidate species for UV studies. Three polycyclic aromatic compounds (PACs) and three oils with varying chemical composition were selected for these studies. UV exposures were conducted using a solar spectrum lamp, and UV dose was measured using both chemical actinometry and a radiometer. Exposure solutions were characterized with GC/MS and total organic carbon pre- and post irradiation. For the single compound exposures, concentrations were maintained using a passive dosing system. For the whole oil exposures, water accommodated fractions (WAFs) were generated using a low energy mixing technique in the dark and under the UV light to simulate the photooxidation that would occur as water interacts with an oil slick.  For the photo-sensitization studies, cod embryos and larvae were allocated into the test solution (single PAC or WAF) and then were left accumulate PACs for 24hrs, after which they were exposed to varying UV doses and then assessed for mortality throughout the subsequent 72 hours. Photosensitization effects were compound, oil, and life stage dependant with embryos being less susceptible than larvae. The photo-modification of single PACs used in this study had no impact on the survival cod embryos or larvae, while photo-modification increased the toxicity of select WAF exposures. The data generated in this study will be used to evaluate the phototoxic-target lipid model and to further our understanding of the impact of UV light during a crude oil spill.

**6.03.P-We187 Recommendations for Improving the Reporting and Communication of Scientific Findings: An Example with Oil Toxicity Testing**

*Adriana Bejarano*[1]*, Julie Adams*[2]*, Judith McDowell*[3]*, Thomas Parkerton*[4] *and* ***Mark L. Hanson***[5]*, (1) Shell Health - Americas, (2) Queen's University, Canada, (3) Woods Hole Oceanographic Institution, (4) EnviSci Consulting, (5) University of Manitoba, Canada*

Results from oil toxicity studies performed with established testing protocols generate data that could be integrated into models and databases that support oil spill planning, response, and environmental assessments. To foster transparency, facilitate repeatability, and maximize use and impact, outcomes from toxicity tests published in the peer-review literature need to be clearly reported and communicated. Thus, the primary motivation of the current work is to provide guidance and encourage more-detailed disclosures of key reporting elements from aquatic toxicity studies. Specific recommendations are provided regarding key reporting elements (i.e., experimental design, test substance and properties, test species and response endpoints, media preparation, exposure conditions, chemical characterization, reporting metric, data quality, standards and statistical methods, and raw data). One outcome of this work is a proposed checklist can be used to assess the completeness of reporting elements or to guide a study design. Improving reporting, science communication, and access to critical information would enable users to assess the reliability and relevance of study outcomes and increased their incorporation into oil spill response tools. These recommendations are also relevant and applicable beyond the oil spill response community, and would ultimately increase the utility of toxicity data in decision-making. This effort represents one contribution of the Modernizing Chemical Response to Oil Spills: Ecological Effects Research Forum (CROSERF) Aquatic Toxicity Testing International Forum.

**6.05 The Cascading Benefits of Green Infrastructure: Chemistry, Ecology & Well-being**

**6.05.T-01 Disturbance Regimes of Stream Metabolism Can be Altered by Green Infrastructure**

***David M. Costello***[1]*, Andrew J. Blinn*[1]*, Anne J. Jefferson*[1] *and Aditi Bhaskar*[2]*, (1) Kent State University, (2) Colorado State University*

Green infrastructure (GI) is placed in urban watersheds with the goal of improving hydrology with expected co-benefits to biological conditions and ecosystem function. While GI can be effective at the scale of individual systems, it is uncertain how the cumulative effect of GI in a watershed can alter storm hydrographs and how that effect can potentially influence stream ecosystem health. To determine the effectiveness of GI at the watershed scale, we examined how primary production and respiration (i.e., metabolism) responded to storm events in three urban watersheds each in Cleveland, OH and Denver, CO. Discharge from USGS gages and high-frequency oxygen and temperature data from sensors were collected in six mid-order streams for two years. Daily estimates of gross primary production (GPP) and ecosystem respiration (ER) were made for each stream using the streamMetabolizer Bayesian model. Storm events were characterized by peak discharge and related to the daily change in GPP and ER using quantile regression. Rates of GPP in Cleveland streams are resistant to high flows below approximately 800 cfs and GPP drops to near zero if storm events exceed this threshold. In Denver streams, the threshold above which GPP was disturbed (100 cfs) was substantially lower than the threshold in Cleveland streams. These threshold responses corresponded with the onset of bed-sediment mobilization which causes scouring of benthic algae. Although GPP was very sensitive to storm flows, ER was not substantially altered by storms of any size in either Cleveland or Denver. Although storms may disrupt benthic heterotrophs, it can also deliver suspended particles and organic matter that may fuel microbial respiration. These data on stream metabolic response thresholds will be included in a Bayesian Network model that will link stormwater management decisions, placement of GI, hydrology, and ecosystem health. These models will be parameterized with a combination of survey data, SWAT models, empirical data, and expert opinion. We anticipate that GI which substantially reduce peak flows will be predicted to have the greatest reductions in disturbance frequency. Overall, our long-term monitoring of hydrologic conditions and ecosystem health is providing key data to better inform our understanding of the cumulative effects of GI at the watershed scale.

**6.05.T-02 Green is the New Grey: Determining the relationships between the biodiversity, soil quality and water balance in urban rain gardens**

***Haley A. Lewis***[1]*, Conor McGarvey*[1]*, Vincent Wang*[1]*, Cameron Moore*[1]*, Murat Akcakaya*[2] *and Kimberly Gray*[1]*, (1) Northwestern University, (2) University of Pittsburgh*

Vegetation is the defining component of green infrastructure (GI) systems as it provides a range of ecological goods and services. Our overarching goal is to determine whether the type of vegetation, native versus non-native plants, matters as it relates to the provision of these goods and services. Over a three-year period, we have studied 15 rain gardens at a total of 9 sites between the Evanston and Pittsburgh area that are paired based on size, age, coverage, and input water source. Using a series of ecological metrics including species richness and conservation coefficients, we have developed classifications for vegetative species quality; high/native, resilient, and non-native/invasive. We find that certain species, although native, can overtake systems and lead to a dramatic reduction in diversity and habitat provision, making it necessary to distinguish between high quality and resilient natives. We have characterized soil quality by measuring indicators of soil health including pH, specific conductance, ion concentrations, organic carbon, nutrients, and total petroleum hydrocarbon concentrations. Since urban rain gardens are perturbed systems receiving polluted runoff with each precipitation event, we investigate how soil quality may influence the diversity, quality, and abundance of the vegetation. We use a variety of multivariate statistical techniques such as principal component analysis, forward feature selection, and support vector machine learning to reduce redundancy in the highly correlated soil data, and to determine the relationship between soil quality and the biodiversity data. Preliminary results show a distinction in soil quality between rain gardens with rooftop stormwater inputs and rain gardens with street stormwater inputs, yet, with no clear distinction in the ecological data between sites. In addition to our ecological and soil assessments, we are studying one site, a long-established rain garden, in greater detail with an installation of intensive sensor arrays to quantify the various components of its water balance (infiltration and evapotranspiration), and to determine if vegetation type (native vs. non-native) influences the water balance.

**6.05.T-04 Hydrological Tools That Expose the Benefits of Green Stormwater Infrastructure for a Non-technical Audience**

***Peter Michael Haas**, Center for Neighborhood Technology*

Municipalities, sewer districts and individual community member have successfully implemented green stormwater infrastructure (GSI) in areas that are vulnerable to urban flooding; engineers, hydrologist, and other technical practitioners have the tools to access the benefits of these GSI implementations. However, to invest and implement GSIs it is often the case that there needs to be a compelling demonstration made that such a strategy would provide the needed control of stormwater runoff and volume capture to reduce urban flooding. In addition to flood mitigation GSI supply a host of associated benefits, such as water quality management, stormwater treatment reduction and habitat restoration; these benefits are not clearly transparent, and they can make the difference in a community's ability convince decision-makers to fund and implement GSI to address flooding issues. The tools used by engineers produce technically detailed estimates of the hydrological effectiveness of stormwater mitigation; these tools however do not readily provide this information and other benefits to a less technical users. The Center for Neighborhood Technology (CNT), The Environment Protection Agency and others have developed tools that allow non-technical users to examine the benefits of GSI in a more intuitive manner. This presentation will explore how they work, what they do and compare their usability and functions including current work at CNT in the development of a GSI Design Hub that includes a more detailed examination of the ancillary benefits of a network of GSI as well as the importance of maintenance.

**6.05.T-05 Use of Sensing-Based Soil Moisture Balances to Track Real-time Performance of a Green Roof**

***Zhaokai Dong**[1], John Buck[2], Daniel Bain[1], Murat Akcakaya[1], Mahmoud Elkhadrawi[1] and Carla A. Ng[1], (1) University of Pittsburgh, (2) Civil & Environmental Consultants, Inc.*

The green roof (GR) is a popular application of green infrastructure to control and manage stormwater in urban areas. To understand its hydrological performance, long-term monitoring may be limited because installation and maintenance of complex monitoring systems are required, depending on the design and size of a full-scale GR. Simulations, therefore, have been attempted to model GR performance and provide insights on the benefits associated with GR implementation. However, an argument in model applications is that the parameterization of GR models (particularly of soil characteristics) may not link to the actual roof physical properties. Low transferability of model parameters among standard models further indicates the uncertainties on the representation of GR structure. Therefore, to characterize key soil properties with respect to hydrological performance of in-place GR installations, we have developed a water balance-based approach using inexpensive and easily installed sensor networks to track the real-time performance of GR. The sensor network used to develop the model includes a weather station to record weather conditions and a simply constructed pan lysimeter instrumented with three soil moisture sensors and a small tipping bucket rain gauge installed beneath to measure drainage. Using the collected data with 5-minute resolution, we assumed 1-D vertical flow throughout the GR and built a conceptual model that focuses on the water retention properties of the GR soil. We divided the soil into 3 layers corresponding to the depth of the three soil moisture sensors. Within each layer the water balance was based on inflow, outflow, evapotranspiration and water storage (represented by the change in soil moisture at each layer). The model successfully predicted the real-time infiltration and percolation rates across the soil profile for long-term monitoring and modeling purposes.

**6.05.T-06 Physics-informed Neural Networks to Analyze the Performance of Green Infrastructures**

***Mahmoud Elkhadrawi**, Zhaokai Dong, Carla A. Ng, Daniel Bain and Murat Akcakaya, University of Pittsburgh*

Our long-term goal is to develop analytical methods to quantitatively analyze the performance of green infrastructures using multimodal data such as rain fall, moisture, volumetric water content, electrical conductivity, chemical soil quality and biodiversity. This abstract specifically focuses on estimating hydraulic conductivity (K) and moisture diffusivity (D) of soil in green infrastructures using multichannel moisture time-series data collected through sensors located at different depths. Our aim is to utilize the changes in K and D across different seasons, years, and weather conditions as quantitative performance metrics for green infrastructures. We follow a physics-informed neural network (PINN) approach to estimate K and D through Richardson-Richards equation (RRE). RRE is a partial differential equation (PDE) that relates the temporal changes in the measured moisture to the spatial changes in the moisture through K and D. PINNs are novel machine learning based architectures that have become popular in the recent years and they use deep neural networks to approximate the solutions of PDEs. In our work we consider the RRE in a single spatial dimension and the neural network parameters are learned through the minimization of the residual of the RRE using the recorded multi-channel moisture time-series data. Such an approach enables us to learn the dynamics of the green infrastructures specifically hydraulic conductivity and moisture diffusivity from the measurement data and known physics. We use the USGS data from Gary Indiana recorded at a green infrastructure located at City Hall. These data are recorded from 07/15/2017 to 7/14/2020. Specifically, we perform peak detection to identify high activity in recorded moisture and use these detected peaks and data recorded within a certain time frame around these peaks for training and testing the PINN for estimation of K and D. Our results are very promising and the mean-squared errors in the training and testing data are negligible. Moreover, as we apply randomization in the initialization of the training set, we aim to achieve consistency in identification of hydraulic conductivity and estimated moisture diffusivity. Our future work will focus on validating the developed approach through lab experiments with experimental columns and further testing the method with data that we are currently collecting from rain gardens located in Phipps Environmental Center in Pittsburgh, PA, and Evanston, IL, USA.

**6.05.T-07 Discussion: Moving from Green Infrastructure to Systems of Green Infrastructure**
***Kimberly Gray***[1] *and Daniel Bain*[2]*, (1) Northwestern University, (2) University of Pittsburgh*
The session leaders will lead a discussion of how to combine the benefits of green infrastructure into more comprehensive systems to harness synergies in green infrastructure interactions and the urban water cycle. This discussion will create enhanced opportunities for interactions between the audience and session presenters (both oral and poster).

**6.05.T-08 Discussion: Evaluation of Evolving Green Infrastructure Systems**
***Kimberly Gray***[1] *and Daniel Bain*[2]*, (1) Northwestern University, (2) University of Pittsburgh*
Session leaders will lead a discussion of emerging tools for the evaluation of green infrastructure function, including wider functions such as patches of biologically diversity, management of invasive species, green space for local communities, and mitigation of storm water impacts. This discussion will create enhanced opportunities for interactions between the audience and session presenters (both oral and poster).

**6.05.P The Cascading Benefits of Green Infrastructure: Chemistry, Ecology & Well-being**

**6.05.P-Tu149 Impacts of stormwater input on ecological quality of restored urban prairies**
***Colleen O'Brien***[1]*, Jennifer Jenkins*[2]*, Vivien Rivera*[1]*, Liliana Hernandez-Gonzalez*[1]*, William Miller*[1] *and Aaron Packman*[1]*, (1) Northwestern University, (2) The Nature Conservancy*
Climate change is leading to more extreme precipitation events in the Midwest, which require new ways of managing stormwater, particularly in urban areas. Green infrastructure has become an increasingly popular way of providing additional stormwater storage, as well as supporting urban biodiversity. However, we don't yet understand the resilience of these ecological systems to inputs of urban runoff, which may carry pollutants that could negatively impact vegetation and accumulate in soils.

Indian Boundary Prairies (IBP) is a prairie-wetland complex located in Markham, Illinois, south of Chicago. The prairies are located adjacent to residential neighborhoods subject to frequent flooding and bordered by two major highways. In 2020, we installed a network of water level and electrical conductivity sensors at two of IBP's prairies, Sundrop (approximately 37 hectares) and Paintbrush (approximately 33 hectares) Prairies. In addition, we collected 54 soil cores from across both prairies. High-frequency water level and electrical conductivity data collected by these sensors provide key information on the hydrologic response of each prairie and areas of the prairie that most frequently receive stormwater input. Using floristic quality indices and analysis of metal and salt enrichment in soil cores as indicators of ecological and soil quality, we analyzed how the ecological and soil quality varies in areas that receive high flow of stormwater with those that do not. Preliminary results show that areas that receive frequent stormwater input tend to have lower ecological quality.

Developing a better understanding of how stormwater impacts the ecology of urban prairies can inform restoration efforts and the design and maintenance of green infrastructure. As green infrastructure becomes increasingly relied upon as a means of stormwater management, understanding how resilient these systems are to inputs of stormwater is critical for the maintenance of these spaces and the services they provide.

**6.05.P-Tu150 Environmental Applications of Additive Manufacturing Featuring Nature-Inspired Design using Natural Materials**

***Alan Kennedy**[1], Andrew D. McQueen[2], Mark Ballentine[1], Zack McCleland[2], Gaurav Savant[2], Chris Williams[3], Burton Suedel[1] and Michael Bortner[4], (1) U.S. Army Corps of Engineers, (2) U.S. Army Engineer Research and Development Center, (3) Virginia Tech, (4) Virginia Polytechnic Institute and State University*

Global waterborne "grey" infrastructure interfaces with the natural environment; yet, design often solely focuses on engineering functionality and misses opportunities to realize natural resource enhancements to achieve additional economic, environmental, and social benefits. Traditional manufacturing often generates geometrically simple structures that do not mimic natural geometries nor are intended to offer multifunctional benefits. However, the design freedom unlocked by 3D Printing (3DP) using diverse synthetic and natural materials is ideal for mimicking natural aesthetics and rapidly testing design prototypes through iterative processes. Therefore, 3DP technologies offer exciting opportunities to rapidly prototype diverse materials and geometries to optimize final infrastructure and artificial habitat performance through progressive design improvements. 3DP could promote use of locally available natural materials (e.g., plant, sand, clay) that offer more environmentally sustainable solutions (e.g., beneficial use of dredged sediments), or addition of materials to existing designs that promote ecological enhancement, contaminant reduction and multifunctional anti-fouling material composites. 3DP habitat and erosion control structures using natural materials promote sustainable infrastructures goals. Examples of environmental applications of 3DP include nutrient sequestration, habitat restoration, erosion control and energy dissipation (e.g., flood risk management). While environmental applications of 3DP have not yet fully been realized, the unique principles and synergistic collaborations available in the Engineering With Nature® community provide the right conditions for a 3DP nature-inspired community of practice to emerge. The objectives are to establish interagency partnerships to brainstorm and unlock the full benefit of 3DP nature inspired-infrastructure for a feasible technology roadmap for scale-up and cross stakeholder benefit, establish process controls and demonstrate use of natural material feedstocks (e.g., beneficial use of dredged material) and to iteratively improve geometrically complex habitat design through hydrodynamic simulation.

**6.06.P Poster Only: Engineering, Remediation and Restoration**

**6.06.P-Tu151 Ionic Strength and Natural Organic Matter Affect Adsorption of Perfluoroalkyl Substances to Colloidal Activated Carbon and its Performance as an In Situ Barrier for Groundwater Remediation**

***Rachel A. Mole**, Adriana C. Velosa and Gregory Lowry, Carnegie Mellon University*

Subsurface injection of colloidal activated carbon (CAC) is an emerging *in situ* groundwater remediation strategy for poly- and perfluoroalkyl substances (PFASs). However, there is still uncertainty regarding long-term barrier effectiveness, particularly in coastal regions with highly variable groundwater conditions. The objective of this study was to consider the adsorption behavior of eight PFASs onto CAC (Intraplex®, Germany) under varying water chemistry conditions. PFASs included perfluoroalkyl sulfonates (PFSAs), perfluoroalkyl carboxylates (PFCAs), and one perfluoroalkyl ether acid. The CAC had a negative external surface charge due to oxygen-containing functional groups but had anion exchange capacity from basic sites on internal pore structures. We hypothesize that shorter-chained PFASs rely on electrostatic interactions in the inner pore space whereas more hydrophobic longer-chain PFASs can adsorb to the internal or external surfaces of the carbon colloids. Batch isotherm experiments were used to probe the effect of increased ionic strength due to either mono- and divalent cations, and the presence of Suwanee River dissolved organic matter (SRDOM). A Freundlich isotherm model was used to analyze adsorption data. High ionic strength conditions led to a decrease in adsorption for shorter-chained PFASs, with the magnitude of effect similar for both mono- and divalent cations suggesting competition for anion exchange sites. Longer-chained PFASs showed minimally enhanced adsorption, due to either electric double layer compression or salting-out effects. In the presence of SRDOM, the adsorption all PFASs decreased, but the magnitude of effect was dependent on perfluorocarbon chain length. Shorter-chain PFASs were much more impacted than longer-chain PFASs. These results suggest a pore-blocking mechanism of SRDOM adsorbed to the carbon surface. Lastly, a basic numerical modeling approach was used to provide a preliminary estimate of time until barrier breakthrough, or when a specified regulatory concentration level was exceeded.

**6.06.P-Tu152 Potential Use of Sulphidated nano Zero-Valent Iron as a Stabilization Agent for Soil Amended with Sewage Sludge**

***Omolola Elizabeth Ojo****, Zuzana Vankova and Micheal Komarek, Czech University of Life Sciences Prague, Czech Republic*

Sewage sludge (SS) is a solid to semisolid material generated from wastewater treatment plants. SS contains a high amount of metals, metalloids, organic pollutants, and other emerging pollutants, which have a direct influence on soil biota. Once treated, sludges are either recycled or disposed of. Recycling SS on land poses a risk of nutrient leaching and impacts soil biodiversity. Although SS is treated before its application to the soil, treatments do not guarantee the complete removal of metals, hence there is a need for effective protection of the natural environment against pollution from sewage sludge. This study aims at investigating the potential use of sulphidated nano zero-valent iron (S-nZVI) as a potential stabilization agent for soil amended with sewage sludge using column experiments, leaching test, and soil pore water analysis.

Five treatments (Control soil, Control + 1% Fe scrap, Control + 1% Composted sludge, Control + 1% S-nZVI, Control + 1% Composted sludge+ 1% S-nZVI) were used in the experiments, with three sets of soil incubation. The first set was used for the column experiment, the second set for the leaching and sequential experiment, and the third set for soil pore water collection. Incubation was done at different time intervals of one day, one week, one month, three months, and six months.

For the column experiment, two different leaching fluids; Synthetic Precipitation Leaching Procedure (SPLP) fluid and Toxicity Characteristic Leaching Procedure (TCLP) fluid were used. After each incubation period, samples were placed into columns connected to an automatic fraction collector. Columns were operated in a down-flow mode at a flow rate of 0.5ml/min. The leachates collected were analyzed for pH, redox potential (Eh), electrical conductivity, organic carbon, and content of metals.

Results obtained so far showed that compost amendment reduced the leaching of metals for samples leached with TCLP fluid in contrast to samples leached with SPLP fluid, where Fe scrap reduced the leaching of metals after long incubation period. Experiments are still ongoing, but the results from the column experiment will be presented.

**6.06.P-Tu153 Water Quality Impacts on Sorbent Efficacy for PFAS Treatment of Groundwater in the Lab and Field**

***Nicholas T. Hayman***[1]*, Jessica Carilli*[1]*, Lewis Hsu*[1]*, Robert George*[1]*, Yina Liu*[2] *and Michael Shields*[2]*, (1) Naval Information Warfare Center (NIWC) Pacific, (2) Texas A&M University*

Per- and Polyfluoroalkyl Substances (PFAS) are a large group of synthetic compounds that have emerged as chemicals of concern in drinking and groundwater. Typically, PFAS-impacted waters are treated to remove PFAS by passing the water through a bed of sorbent material (activated carbon, anion exchange resins [AIX], etc). However, the efficacy of these sorbents varies depending on the types and concentrations of PFAS as well as other water quality conditions such as organic matter content and conductivity. The choice of sorbent material to effectively treat PFAS in complex natural waters will therefore depend upon site water quality and PFAS conditions. To help inform these decisions, we conducted a series of rapid small-scale column tests with two sorbent materials (a granulated activated carbon [GAC] and an AIX), individually and combined, under conditions where conductivity, pH, and organic carbon concentrations were varied in a semi-factorial approach. Artificial groundwaters comprised with these test conditions and spiked with six PFAS compounds (PFBS, PFBA, PFHxS, PFHxA, PFOS, PFOA) were then passed through small columns packed with ground sorbent material for ~30,000 bedvolumes of water for single treatments and ~20,000 bedvolumes for combined treatments, and samples of effluent were captured and analyzed to quantify breakthrough of PFAS from the sorbent materials over time. We found that the tested AIX was more effective than GAC at removing the tested perfluoroalkyl sulfonic acids (PFBS, PFHxS, PFOS), but that GAC was similarly or more effective than AIX at removing perfluorocarboxylic acids (PFBA, PFHxA, PFOA) in many of the test conditions. Overall, the efficacy of AIX at removing PFAS was more strongly impacted by organic carbon and conductivity than GAC, and pH had a more minor effect on either sorbent's efficacy than the other test conditions. In addition, we developed a field-testing apparatus that allowed us to run these column tests in the field to test site-specific groundwater. Data from our field deployment will be discussed. Testing several sorbents at low cost in the field allows for a better understanding of the efficacy of the sorbents given site-specific water quality characteristics, resulting in improved selection of sorbents for pump and treat and/or in-situ remediation.

**6.06.P-Tu154 Stormwater ponds, sinks, or sources of microplastics?**

***Marziye Molazadeh****, Fan Liu and Jes Vollertsen, Aalborg University, Denmark*

Stormwater runoff can be quite polluted as it 'cleans' the city's surfaces of all sorts of dirt and debris. It collects soluble and particulate pollutants, including microplastics (MPs), and conveys them to downstream environments. Concern over the deterioration of downstream watercourses is severe and stormwater is hence commonly treated prior to discharge, for example in wet ponds. However, there is a lack of knowledge on how efficient such systems are towards MPs. To examine this, 13 sediment samples were taken from a stormwater pond in the city of Aarhus, Denmark and analyzed for MPs. The pond receives runoff from residential and commercial areas, and occasionally illicit discharges of wastewater. To further evaluate the retainment efficiency of the pond, a sediment sample was collected from the receiving water, a shallow lake, at the location where the pond discharges.

MPs were extracted in a treatment train applying pre-oxidation, Sodium Dodecyl Sulfate (SDS) treatment, enzymatic treatments, and density separation in a heavy liquid. Extracts were sieved through a 500 µm mesh. MPs below this size were identified and quantified by Fourier Transform Infrared microscopy (µFTIR with Focal Plane Array) at a spatial resolution of 5.5 µm. The obtained hyperspectral image underwent automatic analysis, allowing counting and sizing MPs as well as estimating the mass of each particle. MPs > 500 µm were sorted under a stereomicroscope and all potential candidates analysed for their chemical composition by ATR-FTIR.

The global average concentration across the pond was 11.8 mg kg$^{-1}$ and 44,383 items kg$^{-1}$ of dry sediments. The values for the lake sediments at the discharge point were 0.87 mg kg$^{-1}$ and 7,625 item kg$^{-1}$, respectively, showing a decrease by a factor of 14 when MPs were measured by mass and 6 when they were measured by numbers. Polypropylene (PP) was the most abundant MP type in both water systems. The average PP concentration in terms of mass and number in the pond were 10.14 mg $kg^{-1}$ and 33,727 items $kg^{-1}$, respectively. In the lake at the discharge point, the PP concentrations were 0.55 mg $kg^{-1}$ and 3,500 item $kg^{-1}$, respectively. The results illustrate that MPs, comprising both buoyant and non-buoyant polymers, can be trapped in stormwater pond sediments, highlighting the potential of such facilities as the first barrier in preventing the discharge of MPs to downstream water environments and their role in plastic pollution management.

**6.06.P-Tu155 Congener-Specific vs. Aroclor Analysis of Polychlorinated Biphenyls (PCBs) in School Air**

***Jason Hua*** *and Keri Hornbuckle, University of Iowa*

Airborne polychlorinated biphenyls (PCBs) in school air are emitted from building materials and modern paints and surface treatments, posing significant risk of exposure to occupants. This is especially concerning in schools where children are at risk of greater health effects due to inhalation exposure. Removal of these building materials is an expensive and time consuming process, leading to closure of schools. We hypothesize that there are modern, non-Aroclor sources of PCBs in addition to legacy sources. Further, we hypothesize that congener specific analyses is more revealing than Aroclor analyses. We also hypothesize that there are common sources and profiles between schools. We are using polyurethane foam passive samplers (PUF-PAS) in Vermont schools to measure the prevalence of PCBs in school air. The data generated from this study will be used to determine the prevalence and origin of airborne PCBs in Vermont schools. We will also develop models to predict airborne PCBs as a function of school characteristics. By analyzing PCB profiles throughout a school, we can identify specific sources and develop targeted materials remediation strategies that will help schools save time and money.

**6.06.P-Tu156 Reactivity of Perfluorinated Alkyl Substances (PFAS) on Engineered and Biological Surfaces Using Reductive and Oxidative Processes**

***Hosea Santiago***[1]*, Zimo Lou*[2]*, Susie Dai*[3] *and Gregory Lowry*[1]*, (1) Carnegie Mellon University, (2) Zhejiang University, China, (3) Texas A&M University*

Perfluorinated alkyl substances (PFAS) are a problematic group of chemicals due to their environmental persistence and toxicity. Because of their amphiphilic properties, PFAS can sorb onto many surfaces, whether engineered like granular activated carbon (GAC) or biological such as proteins. For chemical or biological remediation technologies developed to degrade PFASs in water, a critical knowledge gap is whether these compounds react differently when adsorbed onto a nonreactive surface compared to being free in solution. The current study attempts to elucidate this critical consideration by measuring the degradation of a range of PFAS compounds sorbed onto two model engineered carbon materials, granular activated carbon (GAC) and carbon nanotubes (CNT), to determine the effect of adsorption on their reaction rate and product distributions. Because of its promising defluorination efficiencies and quick reaction rates, UV/sulfite-generated hydrated electrons were used for the reductive chemical treatment of PFAS adsorbed to the model carbon substrates. Tracking the PFAS parent compound with LC-MS/MS, fluoride release using ion chromatography, and nontargeted analysis on the final reaction products by Orbitrap MS to complete fluorine mass balances reveal the potential impacts of sorption on the PFAS reaction mechanisms. Initial results suggest that the PFAS desorption rate controls the overall reaction rate, suggesting that these compounds must first desorb and then react free in solution. Additionally, considering that PFAS can sorb onto proteins, an enzyme-mediated oxidative process is studied to confirm whether competing sorption of the PFAS on the protein also controls their reaction rate. This study reveals novel and relevant knowledge about PFAS reactivity on surfaces, which can help develop future sustainable and effective remediation strategies.

**6.06.P-Tu157 Case Study: Successful In Situ Chemical Oxidation at our Iron Ore Canada Sept-Iles site**

***Alexandra Duguay****, Rio Tinto, Canada*

Iron Ore Company of Canada (IOC) operations are integrated across a mine and processing plant in Labrador

City, Newfoundland and Labrador; a port and stockpile in Sept-Iles, Quebec; and a 418 kilometres railway between these two operations

In this case study, we will review the successful implementation of In Situ Chemical Oxidation (ISCO) on more than 18 hectares within our Sept-Iles operation. In brief, it consisted of injecting oxidizing solution in order to chemically break down petroleum hydrocarbons found in soil and groundwater.

With environmental stewardship at heart, a thorough land and groundwater rehabilitation program was designed. Despite COVID, several technical challenges, complex site conditions and constraints, buildings, railways, and underground infrastructures, ISCO resulted in an effective way of remediating contamination from industrial usage of hydrocarbons.

This remediation program supports our commitment to continuous improvement in environmental solutions and implementation of best available technologies.

The Case study will review the following benefits of ISCO:

- treat large contaminated areas without disturbing above ground structures
- elimination of costly excavation and handling of contaminated soil
- the elimination of treatment systems and of capital-intensive equipment and pumps
- reduction in overall treatment time
- Oxidation of dense non-aqueous phase liquids using a combination of catalysts, chelating agents and retarding agents as well as the main oxidizing agent: hydrogen peroxide

The paper will also cover the lessons learned pertaining to design, execution, as well as to many health and safety aspects of our ISCO remediation program. We will provide an opportunity to highlight aspects that could resulted in failures.

We strongly believe sharing the positive outcomes of this program, can support broader use of ISCO, as an important remedial tool for site remediation, particularly in multifaceted large and complex sites such as ours.

**6.06.P-Tu158 Using Localized Surface Plasmon Resonance (LSPR) to Evaluate Proteins as Bio-Sorbents to Remediate Per and Polyfluoroalkyl Substances (PFAS)-Contaminated Water**
***Hajar Smaili*** *and Carla A. Ng, University of Pittsburgh*
Per and polyfluoroalkyl substances (PFAS) is a term that encloses a large number of related fluorinated chemicals that have been used and manufactured since the 1940s. PFAS possess unique properties, such as hydrophobicity, lipophobicity and high resistance to different types of degradation. Thanks to these properties, PFAS are excellent surfactants that have been used in many consumer products such as food containers, personal care products, waterproof textiles, non-stick cookware and aqueous film forming foams (AFFFs) used to fight Class B fires. Because of their potential toxicity and their ubiquity in the environment, PFAS have gained great attention from researchers around the globe, and many efforts have focused on finding effective remediation technologies to treat water bodies contaminated by these substances. Adsorption is one of the most widely used and most practical environmental remediation tools for PFAS, especially long-chain PFAS. Shorter chain substances are more challenging to remove through adsorption because of their lower sorption potential, their hydrophilicity and their high mobility in water. In the present work, we used localized surface plasmon resonance (LSPR) as a tool to assess binding affinity between short chain PFAS of concern and biobased sorbents. LSPR is a label free technique that allows the monitoring of the association and dissociation of ligand-analyte complexes in real time, directly measuring the association and dissociation rate constants ($k_{on}$ and $k_{off}$), in addition to determining the equilibrium dissociation constant ($K_D$). This work uses, for the first time, LSPR

experiments in which biotin-tagged PFAS are immobilized on streptavidin coated sensors to determine their binding kinetics to liver fatty acid binding protein (LFABP). The binding kinetics obtained from these experiments are then compared to the "gold standard" method for assessing PFAS-protein binding such as equilibrium dialysis, to understand the promise of LSPR as a sorbent screening technique.

**6.06.P-Tu159 Biochar Mediated Mercury Transport from a Spiked Soil**

***Geoffrey Millard****, Aaron Betts, Shannon Plunkett, Mark G. Johnson, Chris Eckley and Todd Luxton, U.S. Environmental Protection Agency*

Biochar (BC) is a term applied to a broad category of pyrolyzed carbonaceous material. As a soil amendment, BC has been shown to improve soil fertility for revegetation efforts at contaminated sites as well as potentially sequester organic and inorganic contaminants. The careful selection of biochar would be particularly important at mercury contaminated sites as organic carbon has been shown to either sequester or increase transport of inorganic mercury. A remediation strategy that enhances transport may cause downstream methylation and be counterproductive as methylmercury strongly bioaccumulates and biomagnifies. Using batch reactors, we examine the effects of four different feedstocks (white oak, wheat straw, swine solids and poultry litter) and four pyrolysis temperatures (300°C, 500°C, 700°C and 900°C) on mercury transport from a Hg(II)-acetate spiked garden soil into water. We also separated the effect of the water-soluble fraction and the water-insoluble fraction from whole BC on mercury transport. From the experimental leachate we measured total dissolved mercury, dissolved organic carbon, UV absorption at 254nm, anions, cations, pH and electrical conductivity. Where BC addition produced an effect, generally the water-soluble fraction enhanced mercury transport, while the water-insoluble fraction and whole biochar reduced aqueous transport. Generally, mercury mobilization was reduced at higher pyrolysis temperatures with poultry litter and swine solids performing best.

**6.06.P-Tu160 Unravelling Controls on Plant-Fungal-Metal Interactions in Phytoremediation Contexts**

***Danielle Stevenson****, University of California, Riverside*

A beneficial fungus, arbuscular mycorrhizal fungi (AMF), can potentially enhance phytoremediation by enhancing plant survival in contaminated soils. Though AMF have been shown to mediate their plant partner's interactions with metals, the conditions under which metal uptake is inhibited versus enhanced in AMF-plant partners is unclear; some studies have found that AMF increase metal phytoextraction, while others have found that AMF increase phyto-stabilization.

The study will identify the soil and environmental parameters under which AMF enhance versus inhibit lead, arsenic and chromium uptake into their plant partner and the effect of metals speciation, soil moisture and adsorption on AMF-assisted metal stabilization.

A greenhouse study is being conducted over 16 weeks to complement a multi-year field study on brownfields. Pots are set up using mixed-metal contaminated soil from field sites (brownfields) at two levels: low and highly contaminated and at two soil moisture regimes: droughted and optimal soil moisture. Four dryland phytoremediation plants are being tested: *Erigonum fasciculatum*, *Heteroteca grandiflora*, *Chrysopogon zizanioides* and *Helianthus californicus*. Plants will be grown with and without commercial AMF inoculum and metal uptake into plant roots, shoots and fruits will be tested over time. If possible, bio-XAS will be used to image the location and speciation of the metals within the plant-fungal system.

pH and exchangeable metal concentrations are the soil physico-chemical properties controlling AM fungal-mediated metal translocation to plant partners. Secondly, we hypothesize that translocation of redox-active metals that are mobilized under oxidizing conditions (Cr) will be increased under drier conditions (drought), whereas redox-active metalloids mobilized under reducing conditions (As) translocation into plants mediated through AMF will decrease under wetter conditions. Uptake of divalent cationic metals (Pb and Cd) will be dominantly controlled by pH and carbonate concentrations within soils and therefore have variable response to alterations in soil moisture.

Findings and conclusions will be shared at the conference when the study is complete as it is currently in progress. The findings of this study will be applicable to enhance phytoremediation and also be relevant in agriculture where the potential to inoculate with AMF and inhibit metal-uptake into food crops could benefit public health.

### 6.06.P-Tu161 Performance of Resins and Activated Carbon in Removing Targeted Per- and Polyfluoroalkyl Substances (PFAS) Using Batch Reactor and Pilot Scale Study

***Yaseen Al-Qaraghuli***[1]*, Rominder Suri*[1]*, Mark Fuller*[2] *and Erica R. McKenzie*[1]*, (1) Temple University, (2) Aptim Federal Services, Inc.*

Per- and polyfluoroalkyl substances (PFAS) have been ubiquitously found in the environment, including groundwater. Removing PFAS from groundwater is an acute challenge, and anion exchange resins and activated carbon are promising PFAS treatment technologies. Previous studies reveal that performance is affected by the water matrix, so investigations under environmentally relevant conditions for many PFAS compounds are needed. In this study, activated carbon (Calgon F400) was compared to Purolite anion exchange resins (PFA694E, A592E, USA291597EPF, USA21107, USA212107). Batch isotherm tests, conducted with the six sorptive media and PFAS-spiked groundwater (14 compounds), revealed that the media capacity was generally as follows (from greatest to least removal): PFA694E > USA21107 > USA21217 > F400 GAC > A592E > USA291597EPF. Generally, the removal efficiency was greater for long chain PFAS than short chain PFAS, and for equivalent fluoroalkyl chain, the trend was: perfluoroalkane sulfonates (PFSA) > n:2 fluorotelomer sulfonates (FtS) > perfluoroalkyl carboxylates (PFCA).A pilot scale study at a contaminated site evaluated removal of PFAS from groundwater by the various media under typical use cases: F400 (6 bed volumes (BV)/hr), A592E (16 BV/hr), and USA21107 (16 BV/hr), and PFA694E (60 BV/hr). Within the PFCAs, shorter chain compounds broke through earlier, which was consistent with isotherm findings. Half-breakthrough ($C/C_0 = 0.5$) of perfluorobutanoate (PFBA) was observed as follows: A592E at ~4000 BV, USA21107 at ~8000 BV, F400 at ~9500 BV, and PFA694E at ~12000 BV. Through twelve weeks of use (~14,000 – 160,000 BV), the media completely removed perfluorononanoate (PFNA), indicating good removal of the long chain length PFCA that were detected in the influent. All media removed PFSA compounds, though PFSA influent concentrations were initially quite low ~0.01-0.6 µg/L. The resultant PFAS removal performance for equivalent treated bed volumes was (from greatest to least performance): PFA694E > F400 > USA21107 > A592E. A comparison between the performances of resins under batch study and pilot-scale study is ongoing. Additionally, two resins in the pilot scale have been regenerated, and evaluation of the regeneration effectiveness is underway. In summary, F400 and the resin can remove PFAS from real groundwater and generally have higher capacity for longer chain compounds, though the pilot scale study showed early breakthrough for short chain PFCA.

### 6.06.V-02 Efficiency Assessment of Ferrates to Remove As and/or Mn From Synthetic Neutral Mine Water

***Reem Hussien Safira***[1]*, Lucie Coudert* [2]*, Carmen Neculita*[2]*, Étienne Bélanger*[2] *and Eric Rosa*[2]*, (1) University of Quebec in Abitibi-Témiscamingue, Canada, (2) Research Institute on Mines and Environment (RIME), University of Quebec in Abitibi-Témiscamingue, Canada*

Ferrate [Fe(VI)] can be used as a strong oxidant and coagulant in advanced oxidation processes to effectively remove (in)organic contaminants from synthetic or real industrial effluents. However, Fe(VI) has been scarcely applied for the removal of inorganic contaminants from mine water, despite the large volumes produced, particularly during the exploitation of low-grade deposits. Mine-impacted effluents generally present variable concentrations of metal(loid)s and require performant treatment processes for their simultaneous removal. For example, the co-occurrence of arsenic and manganese in neutral mine effluents, with concentrations up to 20 mg/L, represents a threat to the aquatic life and ecosystems. The toxicity of As and Mn and their complex geochemical behavior in mine effluents, presenting different Eh-pH and Fe concentration dependencies for their removal, highlight the importance and challenge of their simultaneous treatment. In this context, the aim of this study is to assess the efficiency of the Fe(VI) for the removal of As and/or Mn from synthetic and real neutral mine water. Synthetic effluents containing As (3.5 mg/L) or Mn (4.5 mg/L) were treated using different Fe(VI)/As(III) and Fe(VI)/Mn(II) molar ratios, at various pH (5.5 and 6.0 for As, 5.5 and 6.5 for Mn) and retention time in the presence of solid or wet Fe(VI). Based on the results, the Fe(VI)/contaminant molar ratio is a critical parameter, which affects the removal efficiency as well as the residual salinity, while the adjusted pH influences the isoelectric point of the generated sludge, that may affect the sorption of As(V) on the surface of Fe(III) hydroxides. At pH 5.5, complete removal of As or Mn was achieved within the first minute, at Fe(VI)/contaminant molar ratios as low as 4/1 for As(III), and 0.5/1 for Mn(II). The process was deemed effective in quickly removing As and Mn using low amounts of Fe(VI), therefore minimizing the residual salinity and the amount of sludge produced. Further tests are under way to identify the required dose of Fe(VI) for the simultaneous removal of As and Mn from synthetic effluents. A real mine effluent will then be treated under these optimized conditions. Overall, it is anticipated that Fe(VI) could be a promising treatment approach for the simultaneous removal of As and Mn from mine effluents.

## 6.07.P Late Breaking Science: Engineering, Remediation and Restoration

### 6.07.P-Tu191 Graphene-Mediated Removal of Cyanotoxins for Water Security

***Sarah Grace Zetterholm****, Jesse Roberts, Audie Thompson, Angela Evans, Taylor May and Chris S. Griggs, U.S. Army Engineer Research and Development Center (ERDC)*

Water quality can be severely impacted by Harmful Algal Blooms (HABs). One of the main hazards posed by HABs come from cyanotoxins, such as microcystin (MC) and anatoxin produced by various species of cyanobacteria. These toxins, which can also be used as chemical warfare agents, must have a remediation method to ensure water availability and security. Graphene has been investigated for use as a novel adsorbent for a variety of molecular metabolites including these neurotoxins and hepatotoxins. In this study, adsorption isotherms and kinetic studies were conducted to compare graphene performance to traditional water treatment technologies such as granular activated carbon (GAC). Initial results show that graphene removal capacity for these toxins can be up to two orders of magnitude higher than GAC's removal capacity. Additionally, graphene kinetic results show rapid removal of contaminants by reaching equilibrium within 1 hour with pseudo-second-order kinetics. GAC results show much slower removal taking at least 24 hours to reach equilibrium. The removal mechanism for graphene shows evidence of physisorption and can be attributed to a variety of noncovalent interactions such as electrostatic interactions and π-π interactions. This novel materials-based approach for water security can be applied to other cyanotoxins and mitigate a major threat produced by HABs.

### 6.07.P-Tu192 Application of Rapid Small-Scale Column Testing for Media Comparison of Per- and Polyfluoroalkyl Substance Removal

***Margaret Thompson****, David Kempisty and Michael Nickelsen, ECT2*

Per and polyfluoroalkyl substances (PFAS) are a class of chemicals used in manufacturing that are persistent in the environment and resist traditional treatment technologies. Currently, drinking water treatment objectives for PFAS can be as low as single digit ng/L and may be lowered based on the Environmental Protection Agency's 2022 interim updated PFOA and PFOS health advisories. Facilities required to install PFAS treatment systems need to quickly evaluate treatment technologies to meet the changing regulatory landscape. The leading treatment technologies for removal of PFAS are granular activated carbon (GAC) and ion exchange resin (IX). In low concentration drinking water applications, media life can run out to hundreds of thousands of bed volumes before breakthrough of longer chain PFAS such as PFOA and PFOS. Pilot testing of these systems can take months to years and can quickly become cost prohibitive. Batch isotherm tests are quick low-cost options for bench testing but require high PFAS concentrations or large volumes of water for accurate capacity determination and do not capture kinetic impact on system performance. The rapid small-scale column test (RSSCT) can be used to compare media under dynamic loading conditions quickly and with less water than traditional pilot or bench-scale column testing. RSSCTs can provide useful information to predict bed life of both GAC and IX and drive full-scale system design decision making on the basis of cost, footprint, and changeout frequency. Data will be presented from 2 different drinking water sources impacted by concentrations ranging from 50 to 120 ng/L total PFAS. Two GAC and one IX media were tested to simulate full-scale empty bed contact times of 10 and 3 minutes respectively.

**6.07.P-Tu193 Mummichog and Sheepshead Minnow Embryo-Larval Sediment Toxicity Tests: Objective Endpoints for Estimating Sediment Toxicity**

***Alejandra (Alex) Vazquez*[1]**, *Shane Querubin[1], Anna Kate Heider[1], Alfred Pinkney[2] and Lance T. Yonkos[1], (1) University of Maryland, College Park, (2) U.S. Fish and Wildlife Service*

While numerous standardized tests exist for investigating aqueous toxicity in fish, there is no established method for assessing sensitivity of fish to sediment contamination. Reliance exclusively on invertebrate tests and sediment chemistry provides an incomplete picture of environmental harm when making sediment remediation decisions. We modified the existing *USEPA Sheepshead Minnow (Cyprinodon variegatus) Embryo-Larval Survival and Teratogenicity Test* (Method 1005.0) to assess the sensitivity of two minnow species (*C. variegatus* and mummichog, *Fundulus heteroclitus*) to sediment-bound contaminants. Embryos of both species were exposed to dilution series of clean control sediments spiked with reference contaminants representing three classes of legacy organic pollutants (i.e., PAH: creosote oil; Dioxin - 2,3,7,8 TCDD; and PCB: Aroclor 1254), as well as to field-collected sediments containing complex contaminant mixtures. Quantitative test endpoints included: % hatch, time-to-hatch (*C. variegatus* only), and total length. A semi-quantitative endpoint, % normal larvae (incorporating maturity stage at hatch with the ranked presence and severity of developmental anomalies) was also determined. Results of exposures to spiked sediment dilution series produced dose responses with *C. variegatus* more sensitive to creosote oil and Aroclor 1254 and *F. heteroclitus* more sensitive to 2,3,7,8 TCDD. Tests with *C. variegatus* detected quantifiable effects in 21 of 32 (65%) spatially distinct surface sediments collected from two historically contaminated rivers. Frequency of detection of toxicity by test endpoint generally followed the pattern: total length (15) = time-to-hatch (15) > % normal (10) > % hatch (0). *F. heteroclitus* demonstrated differences in detection of toxicity when exposed to a subset of the field sediments mirroring the differences in contaminant sensitivity observed in the spiked sediment tests. These results suggest the two species be used in tandem when investigating sediments with unknown or complex contaminant mixtures. Tests were generally robust with control treatments producing acceptable hatch and growth criteria. However, poor gamete quality or fertilization (volitional or strip-spawned) can influence endpoint interpretation and test acceptability. Detection of toxicity can also be influenced by the source of embryos. For example, in spiked sediment tests, *F. heteroclitus* from a contaminated location displayed reduced contaminant sensitivity.

## 6.07.V Late Breaking Science: Engineering, Remediation and Restoration

**6.07.V-01 The effect of iron on PEDOT performance towards photocatalytic degradation of aqueous organic contaminants**

***Tahereh Jasemi Zad***, *Jenny Malmstrom and Lokesh P. Padhye, University of Auckland, New Zealand*

Conducting polymers (CPs) have been demonstrated as effective photocatalysts for the removal of emerging contaminants in water. The photocatalytic activity of one such promising CP, poly(3, 4-ethylenedioxythiophene) (PEDOT), was probed in this study through a detailed mechanistic investigation. Iron is used as an oxidant or dopant during the polymerization of PEDOT. Although washing the polymerized PEDOT several times has been suggested in the literature for complete elimination of the salt, iron may remain in the matrix of the polymer. This study investigated the effect of residual iron on the photocatalytic activity of

PEDOT. PEDOT was polymerized through an electrochemical process, without the use of an iron source, and a chemical oxidative polymerization technique, using Fe (III).

The photodegradation of a herbicide, hexazinone, an emerging contaminant resistant to photolysis, and methylene blue, a model dye, was tested using Fe (III) and PEDOT individually and in combination under UV light. The results demonstrated an enhanced photodegradation of organic contaminants by an increase in iron concentrations due to the formation of hydroxyl radicals ($^{\bullet}OH$). Moreover, in the presence of electrochemically polymerized PEDOT and in the absence of iron, the degradation of contaminants occurred through a combined effect of adsorption (~53%) and photocatalysis (~75%) at semi-neutral pH after 6 h. The reaction mechanism also showed the important role of $^{\bullet}OH$ in photodegradation.

To investigate the combined effect of PEDOT and iron, two different experiments were carried out. First, Fe (III) was externally added to the PEDOT system, which showed an increase in the degradation of target organic contaminants by the formation of a higher amount of $^{\bullet}OH$. Second, PEDOT strips were dipped in 1 M ferric chloride ($FeCl_3$) solution and washed several times to eliminate unbound iron. However, leaching iron from PEDOT strips into the system was observed. This was confirmed by the immobilized PEDOT through a chemical polymerization process in which $FeCl_3$ was used. Photocatalytic activity of chemically polymerized PEDOT was multifold higher than that of iron-free PEDOT due to the presence of iron. The results of this study showed that UV irradiation can release matrix-bound iron from chemically polymerized PEDOT, resulting in an additive effect of residual iron on the photocatalytic activity of PEDOT.

**6.07.V-02 Identifying Cross Sectional Biotic-Environmental Factors to Maximize Wastewater Denitrification**

***Avni Sharma*[1] *and Jared Kinnear*[2]*, (1) Jesuit High School Portland, (2) Clean Water Services***

Growing scarcity of clean water worldwide requires efficient and sustainable ways to recycle wastewater. A crucial component of recycling is denitrification, which is the removal of harmful nitrates like Ammonium (NH 4 + ), Ammonia (NH 3 ), Nitrogen Dioxide (NO 2 ), and Nitrogen trioxide (NO 3 ) in the water by turning them into harmless gaseous nitrogen (N 2 ). Replicating nature's time-tested sustainable denitrification method requires understanding influential factors for the process and how those influences occur. That knowledge can help operate a fabricated system at the right combination of those factors most efficiently, rapidly, and voluminously. An important experiment for that purpose is described here along with the analysis and results. The experiment was somewhat non-traditional, requiring controlled Design of Experiments (DOE) and naturally occurring happen-stance data that drove combining DOE Analysis of Variance (ANOVA) methods with regression on residuals to fully extract the signal in the available data. Results confirm chemistry-supported influential factors such as carbon source, wastewater flow rate, and temperature, but not pH. Results also highlight the efficacy of Biochar/Woodchip carbon source to enable a large flow rate. This starting experiment crucially explains the influences of the important factors and informs needed future steps for the next series of studies towards the goal of efficient denitrification of wastewater. Data collection by Clean Water Services.

## Track 7: Policy, Management and Communication

**7.01 Bridging the Gap: Teaching Environmental Toxicology and Chemistry in a Dynamic Educational System**

**7.01.T-01 The role of community-based learning in teaching about industrial ecology and sustainability in the context of engineering education A case study from the field**

***Andrea Hicks**, University of Wisconsin, Madison*
Industrial ecology and sustainability education have evolved over time, as this relatively new discipline has emerged and has been refined. Courses engaging with these topics are often found in engineering departments, and feature project or problem- based learning (PBL) commonly with an industrial partner. This provides students the opportunity to apply the topics that they have learned in class in a real-world situation, complete with the constraints and uncertainties inherent in practice. Community- based learning (CBL), a combination of service learning and PBL, allows students to not only apply the material from a sustainability course, but also to do so in service of a community partner. This work presents an analysis of a multi-year effort in teaching a CBL course on the topic of industrial ecology and sustainability in a North American context. Knowledge probes are utilized to quantify student preparation for the course and confidence in knowledge gained during the course in a longitudinal manner. Meanwhile, evolving student reflection is utilized to assess the impact of how the CBL element of the course influenced student thinking about the role of sustainability and industrial ecology within engineering education. The findings suggest that apply- ing CBL to industrial ecology education has the potential to further enhance student learning experiences, while also providing a valuable service experience.

**7.01.T-02 Challenging Students in Critical Thinking with Applied Final Exams**
***Sam Nutile** and Adam Martin Simpson, Penn State Behrend*
Within higher education, courses are designed to introduce students to the specifics of a particular field and generate skills related to critical thinking. Through written assignments and hands-on activities, educators seek to challenge students with tasks that force application of material learned in lecture to specific scenarios. Educators in environmental science benefit from the ability to use lab exercises to demonstrate concepts taught in lecture, but exams can also serve as a viable means of promoting critical thinking. During an upper-level ecotoxicology course, students were given a final exam meant to mimic the process of Environmental Risk Assessment (ERA). The students were first provided with a scenario describing a pond that had been impacted by pollution from manufacturing and agricultural production. Using the information provided in the scenario, the students were asked to answer a series of questions related to the Problem Formulation and Analysis Phases of the ERA. The questions were meant to guide the students in their thought process but challenge them to conceptualize the possible toxicants and routes of exposure that may be related to the adverse effects within the pond. In this process, the students were encouraged to be creative and think critically about their answers without fear of identifying the “correct” answer. Instead, we were hoping the students would develop a relevant conceptual framework and experimental design to conduct sampling in the pond. Once the students submitted the first part of the exam, they were given access to a dataset to complete the Risk Characterization Phase of the ERA. The dataset included sublethal toxicity test data for two chemicals and enzyme activity data related to the chemicals identified. Using the data, the students were asked to complete mixture modeling and calculate effective concentrations related to enzyme activity, then contextualize these data in the context of the adverse effects observed in the pond. Finally, the students provided an overall assessment of risk and a suggestion for mitigating this risk in the future. Overall, the responses received by the students were somewhat underwhelming, but the concept is something we hope to continue to use in the future, after some additional refinement.

**7.01.T-03 Increasing "Learning by Doing" Does Not Mean They Will Do It: Lessons in Project- Based Learning**
***Amanda D. Harwood**, Alma College*
For the past several years, I have been implementing various pedagogical changes to my undergraduate toxicology course. These changes primarily included creating a studio-style classroom and increasing the proportion of project-based assessments. These changes resulted in improved average scores, but perhaps more importantly decreased the range of performance among students. This implied an increased challenge to high achieving students and increased opportunity for lower achieving students. In 2022, the course utilized even fewer traditional forms of assessment (e.g. in class exams), with additional emphasis on projects and reports.

The hope was that this would further improve the performance of both groups of students and continue to reduce the gap among students. This, however, was not the case. This cohort of students had the widest gap in performance despite the greatest opportunity for assistance and reflection on assessments. Several interventions were attempted, including test corrections and reflection activities. Since the majority of students who engaged in these activities were those students who were already successful, this tended to increase their scores, further widening the gap. Students generally had relatively consistent performance across assignment types, indicating that even for assignments with increased time and resources, grades did not improve. For poorer performing students assignments were often incomplete, poorly written, and/or late. This may indicate that this degree of shift to project-based assessments may benefit high achievers without the same positive results for lower achieving students in our current climate.

**7.01.T-04 Choose Your Own Adventure: Incorporating Modular Experimentation into an Ecotoxicology Curriculum**

***Adam Martin Simpson*** *and Sam Nutile, Penn State Behrend*

Because of its broad nature and applications, ecotoxicology can be a challenging field to teach in the classroom. The fundamental tenants of this field—ecology, physiology, statistics, and chemistry—may be harmoniously integrated in practice; however, the intricate nature of these associations is difficult to convey to a diverse audience of undergraduates. Most educators would agree that “hands-on” experiences aid in the presentation and retention of multifaceted concepts, which is why ecotoxicology courses benefit from a laboratory component. Still, the logistics of standard ecotoxicological methods (e.g., animal culturing, toxicity testing, enzyme assays) may not reconcile easily with those of an academic calendar. During the Fall 2021 semester, we adopted a new laboratory approach, designing a “choose your own adventure”-styled project for our co-taught ecotoxicology course at Penn State Erie. Groups of four to five students were tasked with designing ecotoxicological experiments based on a broad “menu” of options that were prepared in advance (e.g., active ingredients, model organisms, experimental conditions, toxicological endpoints, etc.). Additionally, each project featured a secondary objective that augmented their original experiment in such a way that broader toxicological conclusions could be made. After eight weeks the groups presented their data in front of the class for a final assessment. As a whole the class performed well and conceived several intriguing experiments. Student feedback was generally positive, although some students expressed concern about the time commitment and expectations. We did encounter several logistical issues throughout the semester, requiring us to modify our approach. With some refinement, we feel our exploratory lab curriculum encourages second-order thinking in our students, which we hope will bolster their comprehension of convoluted course material.

**7.01.T-05 Estuarine Toxicology: The Tijuana River Estuary Research as a Teaching Model to Promote Community Science Literacy and Student Engagement in Local and International Environmental Issues**

***Maysoon Lehmeidi Dong***[1]*, Armando Vazquez*[2]*, Zuying Feng*[2]*, Lindsey Annette Griffin*[2]*, Flannery McLamb*[2]*, Ken K. Hirata*[2] *and Goran Bozinovic*[1]*, (1) University of California, San Diego, (2) Boz Life Science Research and Teaching Institute*

The Tijuana River Estuary (TRE), the largest coastal wetland in Southern California and the unique and fragile ecosystem, has been a public health hazard and source of contention between the United States and Mexico for decades. Tijuana’s maquila industry has been responsible for water pollution and chemical waste discharges that end up in the estuary and Pacific Ocean. During Covid-19 pandemic (2020/21), The Boz Life Science Research and Teaching Institute and University of California Division of Extended studies piloted an online, two-quarter research-immersion program for pre-college students to investigate the distribution of toxic substances in water and sediments and evaluate embryotoxicity and microbial diversity within the TRE. Sediment and water samples collected from four sites during dry and wet weather events were used in sea urchin embryo toxicity testing; the presence and concentrations of chemical pollutants was investigated by qualitative non-target analysis (NTA) for > 900,000 chemicals in the US EPA CompTox Chemicals Dashboard database using high resolution GCMS and LCMS, and by quantitative targeted analysis of over 150 organic chemicals using GCMS and LCMSMS. Novel passive sampling devices (CIPS) were deployed in water to quantify polar and non-polar organic chemicals, providing a time-weighted-average concentration, while 16s rRNA sequencing of sediment samples was utilized to better understand the correlation between microbial sediment diversity and chemical contamination. 40 pre-college students interpreted relevant scientific literature, generated testing hypotheses, and worked in groups to generate hypotheses and design experiments, apply biostatistics and bioinformatics to curate and analyze the data, and to infer biological relevance. The program concluded with a student group presentation during an annual science symposium attended by scientists, educators, government officials and life science industry leaders. The goal for the TRE research immersion project is to identify public health hazards the estuary and to promote legislative action. The student-led research provides a foundation for continuous community engagement in local and international environmental issues and improves scientific literacy and environmental awareness within the community and beyond.

**7.01.T-06 Empowering Students as Community-Scientists to Investigate Questions in Environmental Toxicology**

***Tawnya L Cary***[1]*, Colleen Flanagan Pritz*[2]*, Katherine H. Ko*[2]*, Colleen Emery*[3] *and Collin Eagles-Smith*[3]*, (1) Beloit College, (2) National Park Service, (3) U.S. Geological Survey*

Student learning is enhanced when instructors apply active learning strategies and engage student in topics relevant to them. Additionally, the COVID-19 pandemic has led undergraduate educators to seek instructional materials that provide students with flexibility and autonomy. For these reasons, I engaged upper-level undergraduates in a community science project investigating mercury bioaccumulation. Since 2011, the Dragonfly Mercury Project (DMP), a joint public engagement effort led by the U.S. National Park Service (NPS) and the U.S. Geological Survey (USGS), has collected and analyzed dragonfly larvae for mercury (Hg) bioaccumulation from >500 sites. From these data, researchers have determined that total and methylHg levels in dragonfly larvae are positively correlated with levels in fish and amphibians, making them effective biosentinels for aquatic systems. Also, dragonfly larvae are easy to capture and identify and are found throughout the U.S.A. making them desirable for community science. Working with NPS and USGS ecologists, I developed a curriculum in which my students made observations from publicly available geometric mean data and generated a question of their own interest (e.g., does proximity to industrial sources influence mercury bioaccumulation?). To aid in hypothesis development, students read primary literature and a lead DMP scientist spoke with our class virtually. Students then requested raw data from specific sites in order to statistically analyze data and test their hypotheses. Additionally, students were invited to be community-scientists and collect dragonfly larvae for the DMP as part of the course. Collecting their own larvae provided them with hands-on experience in the field and highlighted the importance of diligent data collection. To demonstrate their learning, students shared their findings in both written (lab report and infographic) and oral formats. In response to this project, students reported that they increased their understanding of how science works ("science is a real thing conducted by real people"), appreciated science more and how it can relate to the general public, and 83% of students (n=6) indicated that they are more confident in their ability to "think like a scientist". Community-science projects are an effective strategy to engage students in asking questions pertinent to their interests, while providing them the opportunity to contribute to an authentic scientific endeavor.

**7.01.T-07 Teaching Mathematical Models with Interactive Software Tools**

***Ian Moran***[1]*, Emily Bonner*[1]*, Steven O'Connell*[1,2]*, Diana Rohlman*[1] *and Kim Anderson*[1]*, (1) Oregon State University, (2) MyExposome, Inc.*

One challenge in teaching and science communication is conveying the structure and function of mathematical models. Models are widely used in environmental toxicology and chemistry to describe contaminant fate, dose response relationships and risk. Interpretation of models and their strengths and weaknesses is often limited by one's direct experience in their use, posing a challenge to students and non-specialist model users with infrequent applications. Interactive web-based applications created from open-source software tools provide a means for students to engage in hands-on experimentation with mathematical models, without a background in

coding. These student-centered tools can enable users to visualize the inner workings of complex models through an approachable framework, adding depth to their understanding. For example, in a model that relies on ambient temperature and humidity, the student can increase or decrease each variable to determine the effect of each on the outcome. This self-directed exploration builds an intuitive understanding otherwise requiring extensive experience. Here we demonstrate the use of these tools for diverse audiences, from middle and high school students to career scientists. First, we describe the use of web-based applications to convey the structure of chemical transport models to professional scientists and regulators during a virtual workshop hosted by Oregon State University's Superfund Research Program. Second, we describe the use of these tools in outreach activities with underrepresented pre-college students to generate dose response curves and point estimates from their experiments. Feedback from workshop participants and educators reflected that these tools are effective and well suited to asynchronous educational activities and remote-learning during the COVID-19 pandemic. These examples demonstrate the utility of web-based visualization tools to teach mathematical models to diverse audiences, and to enable the use of powerful software tools without the need for specific training in coding.

**7.01.T-08 Panel Discussion: Teaching Environmental Toxicology and Chemistry in a dynamic educational system**

***Sam Nutile***[1]*, Corinna Singleman*[2]*, Adam Martin Simpson*[3] *and Amanda D. Harwood*[4]*, (1) Pennsylvania State University, (2) Queens College, (3) Penn State Behrend, (4) Alma College*

Education in environmental toxicology and chemistry involves engaging students during lecture and laboratory courses with information that is relevant to the field of study. Developing exercises that deliver the appropriate material that is accessible to students from a variety of experiences , however, can be difficult. One of the best ways by which to develop new pedagogical methods is through sharing ideas with other educators. Therefore, the objective of this panel discussion will be to provide an opportunity for participants to discuss techniques used in the classroom to engage students of various academic levels in topics related to environmental chemistry and toxicology. Using the information discussed in the preceding platform presentations, the co-chairs will lead a discussion on laboratory exercises, exam formats, writing assignments, and online tools that can be used to better involve students in and out of the classroom. All participants will be encouraged to share ideas of what has and has not worked in the past with the goal of brainstorming new ideas. Through this collaboration, we hope to inspire new pedagogical techniques to be deployed in the coming year.

**7.01.P Bridging the Gap: Teaching Environmental Toxicology and Chemistry in a Dynamic Educational System**

**7.01.P-Tu162 DataScience4 - Environmental Science: An interactive classroom ready resource where students learn by exploring expert-curated environmental datasets**

***Ryan R. Otter***[1]*, Zach Gemignani*[2]*, Cheri Burt*[1] *and Alex Murphy*[1]*, (1) Middle Tennessee State University, (2) Juice Analytics*

Students learn by doing, not watching. A major hurdle for educators, especially in science, is the development of hands-on exercises that put students in control of their own learning, rather than being an observer. Dissecting a frog or mixing chemical for colorimetric reactions are excellent ways for students to directly engage with the material they're learning, but what about topics that don't lend themselves to hands-on experiments?  Problems like species conservation, climate change, or contaminant concentrations?  These types of problems need a different type of hands-on learning system, one based in data exploration. To directly address this problem we created a free, publicly available classroom-ready learning environment where students can explore expert-curated environmental datasets. In this presentation we will present the approach we took to develop DataScience4, the resources that exist for teachers, and show the technology that powers our platform.

**7.01.P-Tu164 Make STEAM Q: Using the 'Making' Mindset to Improve Student Outcomes**
***Corinna Singleman**, Matt Greco and Nathalia Holtzman, Queens College*
The goal of the Make STEAM Q project is to create a community of faculty from different disciplines who each integrate "making and design thinking" activities in their undergraduate courses. Faculty fellows come from Hispanic-Serving four-year Queens College and partner with the New York Hall of Science (NYSCI), a science museum in Queens, NY. The Make STEAM Q team works with faculty fellows to foster the development of maker mindsets to (re)invigorate their diverse students' interest and participation in STEM with the support from NYSCI and Queens College's Makerspace.

The faculty fellows develop courses or course modules incorporating making and design thinking utilizing the tools and physical space in the Queens College Makerspace. Through formal and informal learning they integrate a Design-Make-Play framework to build a community of practice through best practices in andragogy, i.e. ways to teach adults. Throughout the project's term, fellows participate in professional development sessions addressing faculty attitudes, teaching expectancies and readiness to incorporate design-make-and-play thinking approach into their courses.

Along with building a supportive group of faculty at a difficult time in education, recent professional development meetings engaged faculty in discussions on how to incorporate making practices in exclusively online learning environments. Throughout the pandemic the collaboration between NYSCI and QC fostered creative solutions to making in the virtual learning environment. One long-term goal of the Make STEAM Q project is to not only incorporate the depth of experiences and knowledge from NYSCI into Queens College, but in turn, to send faculty and students to NYSCI to share their own experiences and STEAM knowledge with the NYSCI and Queens community at large.

**7.01.V Bridging the Gap: Teaching Environmental Toxicology and Chemistry in a Dynamic Educational System**

**7.01.V-01 Designing comics as a way to learn environmental toxicology**
*Tiare Huizar-Hernández and **Patricia Ramírez Romero**, Universidad Autónoma Metropolitana, Iztapalapa, Mexico*
Due to the COVID pandemic senior students had to look for different ways to develop research to obtain their B. Sc. degree. This study presents the results of a comic that was designed to present information regarding emerging contaminants (farmaceuticals) to highschool students. The design of the comic considered the public to target, the scientific information related to farmaceuticals as pollutants as well as that of the chemical techniques used to identify and to quantify these substances. A real location was chosen to develop the story as well as the characters. The story takes place in a coastal locality of the Gulf of Mexico where children find dead animals. They want to know why this happened so they get in touch with an Ecotoxicologist. She develops an investigation and shows the results to the town in a public gathering where she not only explains the problem but also the solutions. The comic´s impact was tested with highschool students who were polled after reading it. The majority understood the topic and learned new scientific information throught it. We concluded that comics are a good teaching tool for all ages.

**7.02.P Defining Environmental Justice in Context of Impact and Outcomes**

**7.02.P-Mo175 Engagement to determine the key issues and obstacles to stakeholder and community uptake of an urban geothermal resource**
***Michelle Bloor** and Ewan Gibbs, University of Glasgow, United Kingdom*
Glasgow is a city where around a quarter of households live in fuel poverty. The city and surrounding areas have a long association with industry and mining. The aim of this project is to develop effective stakeholder

narratives to accelerate a sustainable deployment of urban minewater resources while enabling a just transition and a timely UK supply chain around minewater geothermal as part of the ongoing energy transition to net zero. The project will identify potential ‘impacts’ as a complex *wicked* policy issue and draw on Bacchi’s (2009) “*What’s the Problem Represented to Be?*” approach to policy and practice analysis, as well as similar stakeholder consultative work by Kivitis (2011) and KIzydorczyk et al (2019).

A systematic review will initially be undertaken, upon which an inventory of the potential ‘impacts’ will be created, including capacity, ownership of heat and governance. An expert workshop will be held where we will hear from academic, government and industry experts to get balanced opinions on the topic and a briefing paper will be generated. A ‘stakeholder mapping’ exercise will take place, which includes focused engagement with a diverse range of ‘relevant’ stakeholders. Using the briefing paper as a foundational base, the of the nature of *problems* will be scoped.

Community engagement activities will be undertaken, which build on research about energy citizenship in the context of climate change. It will focus on the potential for geothermal energy to develop ‘prosumer’ relationships through making consumers engaged agents influencing localized forms of energy production. Community engagement revolved around a travelling and on-line digital exhibition, images of the past that link to coal mining and related economic activities as well as maps of mine workings will be used to illicit responses from members of the public within the six targeted geographical locations. The fora will provide opportunities to engage with the local communities, to hear their views on past coal mining activities, and feedforward by profiling the potential that geothermal heating systems offer communities considering climate change and energy price instability. During the exhibition, written feedback walls will be dedicated to ask local communities their views on former mine workings and their expectations from geothermal.

## 7.03 Deriving and Implementing Ecologically Relevant Water Quality Criteria and Guidelines

### 7.03.T-01 Considerations for Selecting and Adopting International Ecological Screening Values
***Sagar Thakali****, Heather Loso, Catherine Schwach, Belinda Goldsworthy and Michael Archer, AECOM*
Protection of flora and fauna is an integral part of the world-wide sustainability goals. Existing ecological screening values (ESVs) from various western countries are being adopted world-wide to screen for chemical risks to ecological receptors. Several factors need to be considered in these adoptions, including but not limited to the relevance of the underlying species to specific countries/regions, the level of species protection sought for specific land uses, the confidence in the existing ESVs, and local/regional background levels.

We have compiled and reviewed the existing ESVs for surface water from Australia, Belgium, Canada, the Netherlands, and the United States. We have developed a methodology to rank-score multiple ESVs for a chemical based on the ESV derivation methodologies (e.g., species sensitivity distributions, assessment factors, or other extrapolations) and underlying database (species/taxa represented, acute vs. chronic data, etc.). Ultimately, we have developed an approach to select and adopt ESVs based on country-specific needs and relevance, such as different levels of species protection for different areas/land uses and representation of prevalent and/or protected species.

In this talk, we will provide key aspects of our critical review of the existing ESVs, the methodology to rank-score multiple ESVs for each chemical, and further country-specific considerations we took for their selection and adoption. Our study and findings will provide a technical framework to consider in future selection and adoption of existing ESVs in different countries.

### 7.03.T-02 Quantifying Conservatism in ecoTTC and CTD: Case Study of Benzene-Like Chemicals with Regulatory Water Quality Criteria Values

***Kristin A. Connors**[1], Adriana Bejarano[2], Constance Mitchell[3] and Michelle Rau Embry[3], (1) Procter & Gamble, (2) Shell Health - Americas, (3) Health and Environmental Sciences Institute (HESI)*
Water quality standards are used globally to assess water quality impairment and for the protection of aquatic life. Typically, these water quality values are based on aquatic toxicity data and species sensitivity distributions (SSDs) or other probability distribution-based approaches on a chemical-by-chemical basis. There is increasing need to evaluate and set criteria values for a large number of chemicals via international regulatory mandates, but with reduced reliance on vertebrate testing and limitations on available resources. Grouping approaches based on use of existing knowledge can help to fill this gap and potentially allow for criteria value derivation across a broader chemical space. The EnviroTox database is a curated compilation of aquatic ecotoxicology data for over 4200 chemicals with associated physical chemistry data, mode of action (MOA) assignments, and detailed taxonomic information. These data can be used with the integrated online tools to calculate Predicted No-Observed Effect Concentrations (PNEC) for each chemical by applying the relevant assessment factors (according to US or European-based regulations). Statistical distributions can be created using either toxicity hazard values or PNECs, creating a chemical toxicity distribution (CTD) or ecological threshold of toxicological concern (ecoTTC), respectively. These two approaches enable toxicity prediction for untested chemicals based on chemical grouping according to MOA or structural features and have potential to inform water quality criteria derivation. A case example of non-chlorinated benzene-like compounds was developed to explore the relationships between existing aquatic toxicity-based water quality standards and eco-TTC and CTD estimates. This presentation will provide an overview of the case study, the approaches utilized, rationale for group selection, and a quantitative comparison of the degree of conservatism in ecoTTC and CTD approaches. Guidance will be provided to new users of this tool to explore case studies and applications to chemical evaluation strategies.

**7.03.T-03 Deriving Environmental Quality Standards for fipronil under the European Water Framework Directive for UK freshwaters.**

***Graham Merrington**[1], Dean Leverett[1], Lucy Kennelly[1], Adam Peters[1] and Helen Wilkinson[2], (1) wca environment limited, United Kingdom, (2) Environment Agency, United Kingdom*
The insecticide fipronil is currently authorised for use in the UK as a veterinary medicine for the treatment of fleas and ticks on cats and dogs and as a biocide. The derivation of an Environmental Quality Standard (EQS) has been prompted by the recent identification of potentially toxic fipronil concentrations in UK freshwaters, alongside its high potential hazard to aquatic life. The ecotoxicity of fipronil has been assessed and to protect against long and short-term exposures and potential impacts upon wildlife have been derived. Fipronil has a mode of action that infers greater toxicity to insects and related species than other organisms, and the available ecotoxicity data generally support this view. There are considerable amounts of ecotoxicity data for freshwaters, but a great deal less for marine waters. Some of these data are openly available, others are owned by industry groups and are proprietary. Here we attempt to use all the available data, generated either in the laboratory or mesocosms, and following the current European Technical Guidance derive annual average (chronic) and maximum acceptable concentration (acute) standards.

There is a large dataset of reliable and relevant acute ecotoxicity data covering 48 species from 10 higher taxonomic groups. There is a much smaller dataset of chronic toxicity data, although the size is increased considerably by combining this with marine data, given the mode of action of fipronil this approach is questionable. Where possible probabilistic approaches have been used to derive EQS values, and the uncertainties surrounding these are considered in the derivation of the values of the EQS. Overall, the complete datasets for both acute and chronic toxicity show a very poor fit to a log-normal distribution, and although some other distributions do provide a better fit to the datasets none of those considered provided a good description of the lower portion of the distributions from which the EQS is derived. A dataset based on the most sensitive taxonomic groups, insects and crustacea, for acute toxicity does provide a considerably improved fit to a lognormal distribution, and potentially allows for a satisfactory derivation of the hazardous concentration for 5% of these sensitive species.

**7.03.T-04 Updating the Chronic Nickel Biotic Ligand Model for Regulatory Applications in Europe**

***Adam Peters***[1]*, Charlotte Nys*[2]*, Graham Merrington*[1]*, Elizabeth Middleton*[3] *and Christian E. Schlekat*[3]*, (1) wca environment limited, United Kingdom, (2)A RCHE Consulting, Belgium, (3) NiPERA, Inc.*

Nickel was identified as a Priority Substance in Europe and a generic risk assessment was conducted and subsequently a continent-wide Environmental Quality Standard (EQS) was set for chronic freshwater toxicity. The EQS for nickel was set as a "bioavailable nickel" concentration to ensure that a consistent level of protection was afforded to different types of surface waters. The chronic Biotic Ligand Model (BLM) for nickel was developed for the generic risk assessment is used as the basis for assessing compliance against the EQS for nickel and enables the entire ecotoxicity database to be normalised to the bioavailability conditions of local compliance assessment sites. When applied in this way the BLM is effectively the integration of several species-specific bioavailability models with a comprehensive ecotoxicity database through a procedure for applying the individual bioavailability models to all of the different kinds of aquatic biota that are included in the database. This integrated approach enables site-specific thresholds to be calculated for local water chemistry conditions. It is over a decade since the EQS was set and a significant quantity of additional information concerning both nickel toxicity to aquatic organisms and the bioavailability of nickel under sensitive water chemistry conditions has become available during this time. This presentation provides a summary of updates that have been made to both the ecotoxicity database, the bioavailability models, and their integration to provide site specific thresholds for nickel. A total of 152 toxicity data covering 32 species from 28 different studies have been added to the existing database, and this included 24 additional species that were not previously represented. The updated ecotoxicity database includes 360 tests covering 55 species, all of which can be normalised for the local bioavailability conditions. The bioavailability normalisation procedure has also been updated to include updates to the bioavailability models that enable more sensitive water chemistry conditions to be covered by the model predictions. The updated database and bioavailability normalisation procedure are applicable to more than 95% of European surface water conditions and provides an extremely robust basis for the derivation of regulatory thresholds for chronic nickel toxicity in freshwaters such as Predicted No Effect Concentrations and EQS and is protective of the results of several mesocosm studies.

**7.03.T-05 Evaluating the Benefits of Improved Implementation and Revision of the Environmental Quality Standard for Nickel under the Water Framework Directive**

*Adam Peters*[1]*, Elizabeth Middleton*[2]*, Graham Merrington*[1] *and* ***Christian E. Schlekat***[2]*, (1) wca environment limited, United Kingdom, (2) NiPERA, Inc.*

Nickel has been identified as a Priority Substance in Europe and the European Commission set a continent-wide Environmental Quality Standard (EQS) for nickel in freshwaters under the Water Framework Directive in 2013 that was based on the "bioavailable nickel" concentration. This standard has been implemented to varying different extents by different European Member States because of a lack of suitable guidance or sufficient familiarity with the tools required. The European Commission published guidance on the implementation of bioavailability-based standards in late 2021 and conducted a revision of the EQS in order to update it in light of new scientific data that was finalised earlier this year. This presentation considers the relative benefits of maintaining the existing EQS, but with an increased focus on its proper implementation, versus updating and revising the EQS to take account of new scientific developments. Both approaches have potential benefits in terms of protecting the environment from potential problems due to emissions of nickel. Improved implementation of the existing EQS would build upon the experiences already gained, especially by those Member States that have implemented the bioavailability-based standard effectively and would identify potential problems whether they are due either to nickel sensitive water chemistry conditions or elevated nickel exposures. Updating the EQS would enable recent scientific developments to be taken into account, and a key one of these is the ability to assess nickel bioavailability in more sensitive high pH waters than is possible based on the existing EQS. As the EQS for nickel is set on a Europe-wide scale it is important to consider the overall situation throughout Europe as regards potential nickel toxicity in freshwater systems to provide context to the situation and the potential benefits offered by the different possible approaches. Based on an indicative compliance assessment for a number of countries for which suitably detailed information can be obtained it would appear that levels of compliance against the existing EQS are likely to be very high where it is properly implemented as a bioavailable nickel concentration, but there also appear to be a number of countries where nickel bioavailability assessments should be conducted but the additional information required to do so is currently not available, highlighting limitations in the current implementation.

**7.03.T-06 The structure of aquatic life criteria definitions for time-varying conditions: Arguments for replacing averaging periods with allowable frequencies of exceedence**

***Chris Mebane**, U.S. Geological Survey*

Since 1985 in the United States, aquatic life criteria for chemicals s (ALC) typically follow a 2-number form to address time-varying concentrations: an acute criterion to protect against brief, episodic events and a chronic criterion to protect aquatic communities from long-term, stable exposures to pollutants. In turn, both the acute and chronic criteria expressions have 3 terms: an allowable magnitude (concentration), duration, and an allowable return frequency of exceedences. For example, at a water hardness of 60 mg/L as $CaCO_3$, aquatic life should be protected from dissolved cadmium (Cd) toxicity if the 4-d average concentration does not exceed 0.5 µg/L more than once every 3-y on the average (the chronic criterion) and likewise, if the 1-hour average concentration does not exceed 1.1 µg/L (the acute criterion). These 1-h and 4-d durations were based on toxicology with no consideration of feasibility for monitoring and assessment. Because some state water-quality assessment programs interpret grab samples to represent 1-h average concentrations but seldom have data frequent enough to represent 4-d averages, this effectively means that criteria attainment judgements will be based on acute criteria exceedences only. Therefore, in practice, criteria as implemented may provide substantially less protection than intended, as data sufficient to interpret the lower, chronic criterion concentrations may never be obtained.

This 1-h and 4-d averaging period approach will be contrasted with an alternative exceedence frequency approach. In this approach, water bodies would be considered compliant with criteria if 95% of monitored values over a 3-y period were within criteria and no value was greater than 2X the criterion value. The approaches are contrasted using real data from diverse monitoring records. Simulations where the magnitude of exceedence is projected against the potentially affected fraction (PAF) of species sensitivity distributions (SSDs) indicate the frequency exceedence approach would meet objectives of protecting aquatic life communities. The 'no value greater than 2X the criterion' provision was an explicit assumption in the protectiveness of the 'once in 3-y' allowance for exceedences but was never explicitly included in criteria definitions. Whether 2X is the most appropriate limit on allowable magnitude of exceedences would require further thought and, for example, acute-to-chronic effects ratios might be more suitable.

**7.03.T-07 Discussion 1 of 2: Water Quality Criteria and Guidelines**

***Adam Peters**[1], Chris Mebane[2] and Jennifer Stauber[3], (1) wca environment limited, United Kingdom, (2) U.S. Geological Survey, (3) CSIRO, Australia*

The 30-minute discussion block is intended to allow open discussion on topics related to the session. In particular, some of the ideas in the session talks may novel or controversial and would benefit from discussion. We welcome comments relating to the following questions:

How should the substances requiring water Quality Criteria be identified and prioritised?

1. How can we evaluate whether a dataset covers sufficient diversity, either in terms of the taxonomic groups or endpoints represented within it, and do the species included need to be relevant to the region that the resulting criteria is to be applied in?
2. How can issues of complexity and ecological relevance, such as bioavailability, be included in criteria derivation and implementation?
3. Should field and mesocosm evidence be used for criteria derivation or is it only suitable for assessing the reliability and validity of criteria?
4. How can we ensure that the criteria derived are fit for purpose and will enable users to implement them in practice?

**7.03.T-08 Discussion 2 of 2: Water Quality Criteria and Guidelines**

***Adam Peters**[1], Chris Mebane[2] and Jennifer Stauber[3], (1) wca environment limited, United Kingdom, (2) U.S. Geological Survey, (3) CSIRO, Australia*

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3. Should field and mesocosm evidence be used for criteria derivation or is it only suitable for assessing the reliability and validity of criteria?
4. How can we ensure that the criteria derived are fit for purpose and will enable users to implement them in practice?

**7.03.P Deriving and Implementing Ecologically Relevant Water Quality Criteria and Guidelines**

**7.03.P-Th142 Evaluating the Relevance of an Environmental Quality Standard for the Secondary Poisoning of Aquatic Predators by Nickel**

***Adam Peters**[1], Graham Merrington[1], Elizabeth Middleton[2] and Christian E. Schlekat[2], (1) wca environment limited, United Kingdom, (2) NiPERA, Inc.*

Nickel is a Priority Substance in Europe and consequently a continent-wide Environmental Quality Standard (EQS) was set for chronic freshwater toxicity under the Water Framework Directive. The EQS for nickel was set as a “bioavailable nickel” concentration to ensure that a consistent level of protection was afforded to different types of surface waters, representing the best available scientific understanding of nickel toxicity to aquatic ecosystems at the time. Almost a decade after the existing EQS was set, and even longer since the ecotoxicity database and models used were finalised, the European Commission has recently reviewed the EQS for nickel to update it in light of new scientific data and to the align it with updated technical guidance. The conclusions of this review considered the secondary poisoning of aquatic predators to be a potentially important tissue for nickel, and concluded that the existing EQS for the direct toxicity of nickel to aquatic ecosystems base don the direct toxicity of bioavailable forms of nickel would not be protective of sensitive aquatic predators such as otters. This presentation identifies a tentative threshold for nickel accumulation in the livers of mammals from food sources based on feeding studies with other mammals. This threshold is then compared against existing data for the concentrations of nickel in the livers of otters that has been reported from a collection of animal carcasses from the UK. Information on both historic and current nickel exposures in UK surface waters is also used to provide context to the conclusions drawn about the potential for otters to be exposed to excessive levels of nickel via their diet.

**7.03.P-Th143 Multi-step Integration of Ecotoxicological Study Reliability in Ecological Risk Assessment**
*__Stephanie B. LaPlaca__, Melissa M. Heintz, Daniele Wikoff and Laurie C. Haws, ToxStrategies, Inc.*
Evaluation of study reliability is a critical component of toxicity value development, as well as in the overall risk assessment process. As approaches vary and are evolving with the use of systematic review, the objective herein was two-fold: (1) to conduct a survey of how ecotoxicology study reliability is assessed by authoritative bodies globally, and (2) to propose a workflow to objectively integrate study quality considerations in the development of PNEC values for use in ecological risk assessment. The survey demonstrated that many regulatory agencies use Klimisch or Criteria for Reporting and Evaluating Ecotoxicity Data (CRED) methodologies; in practice, however, these frameworks are frequently modified and are utilized in differing capacities in the risk assessment process. In addition, different offices within the USEPA have their own guidance and approaches. Unique and overlapping criteria were identified in each study quality tool; these attributes were subsequently utilized in the proposed workflow. Specifically, we propose a tiered assessment of study reliability which involves a first step of assessment of minimum reporting and conduct standards to be considered for inclusion (e.g., test substance identification), similar to that employed by the USEPA for the ECOTOX database. Second, critical appraisal of reliability using a refined technique in which CRED criteria were modified to better incorporate systematic review principles. The proposed tool is modeled after the National Toxicology Program (NTP) Office of Health Assessment and Translation (OHAT) guidance, created to place emphasis on key criteria and formulation of an overall tier for reliability in toxicity value derivation. Key categories given the most weight that are incorporated into this tool include: i) are appropriate control groups tested and their performance reported? ii) is the performance of the exposed test species evaluated, including observations for toxicity? iii) is the exposure method, route, frequency of administration, and length of treatment period reported and consistent across study groups? This workflow and revised critical appraisal tool incorporates existing evaluation methods used across regulatory agencies in addition to systematic review principles, providing a thorough and transparent study quality review process that can be universally applied in assessing studies for toxicity value development and use in risk assessment for ecotoxicological endpoints.

**7.03.P-Th144 Application of an Integrated Ecotoxicological Study Reliability Tool in the Derivation of Predicted No-Effect Concentrations for Short Chain and Ultrashort Chain Per- and Polyfluoroalkyl Substances**
*__Melissa M. Heintz__, Stephanie B. LaPlaca and Laurie C. Haws, ToxStrategies, Inc.*
A critical part of PNEC (predicted no-effect concentration) development is the assessment of ecotoxicological studies for reliability prior to the identification of relevant points of departure (PODs). A novel tool for the critical appraisal of study quality that integrates components of existing reliability assessment frameworks for ecotoxicological studies from various regulatory agencies with systematic review principles was employed to derive acute and chronic PNECs for aquatic toxicity for emerging short chain and ultrashort chain per- and polyfluoroalkyl substances (PFAS). First, a pilot study was conducted using the new tool to test its utility, as well as the reproducibility of results between reviewers by evaluating the aquatic toxicity studies identified for a single ultrashort chain PFAS compound - trifluoroacetate (TFA). A total of 7 relevant publications were identified from the peer reviewed literature, along with 3 unpublished reports. Each of these studies were assessed by multiple reviewers using the novel study reliability tool. Conclusions on reliability of each study were congruent across reviewers, resulting in a total of 6 studies included for subsequent toxicity data extraction. Based on the reproducibility achieved in the initial pilot of the tool, the reliability of aquatic toxicity studies identified for additional ultrashort and short chain PFAS was then assessed using the integrated critical appraisal tool, and relevant toxicity data were extracted from adequate studies. For each PFAS, PODs for relevant endpoints were evaluated using a Weight of Evidence (WOE) approach to select acute and chronic PODs representative of the underlying toxicity data. PNECs were derived from selected PODs using

appropriate uncertainty assessment methods (i.e., assessment factors or sensitive species distribution). Calculated PNECs were then compared to existing toxicity values reported in the literature or to those derived by regulatory bodies to determine how application of the integrated ecotoxicological study reliability tool affects the final toxicity value. Initial findings from this work demonstrate the tool's utility and reproducibility. Importantly, this tool will increase confidence in aquatic toxicity values developed for emerging short chain and ultrashort chain PFAS for ecological risk assessment purposes.

**7.03.P-Th145 Development of U.S. Perfluorooctanoic Acid (PFOA) Ambient Water Quality Criteria for Aquatic Life**

***James Justice****, Amanda Jarvis, Brian Schnitker, Mike Elias and Kathryn Gallagher, U.S. Environmental Protection Agency*

Perfluorooctanoic Acid (PFOA) is part of the broader group of Per- and Polyfluoroalkyl Substances (PFAS). Given its unique physiochemical properties, PFOA has been used in a wide range of industrial and consumer products. Following use and disposal of these products, PFOA may be released in the environment where it is highly persistent, being detected in water and organismal tissues within aquatic ecosystems. In accordance with the U.S. Environmental Protection Agency's PFAS Strategic Road Map, the Agency is developing national PFOA Water Ambient Quality Criteria for freshwaters and benchmarks for estuarine/marine waters to protect aquatic life from elevated PFOA exposures in ambient waters. The draft PFOA Aquatic Life Criteria document was released for a public comment period in April 2022. The goal of this presentation is to provide an overview of the PFOA freshwater criteria and estuarine/marine benchmarks, describe the toxicity studies used to derive the draft PFOA criteria and benchmarks, and summarize the scientific comments received from the public. Once finalized, the PFOA criteria will reflect the maximum concentrations, with associated frequency and duration specifications, that would support protection of aquatic life from acute and chronic effects associated with PFOA in freshwaters.

**7.03.P-Th146 Development of Aquatic Life Ambient Water Quality Benchmarks for Data-Limited PFAS Using New Approach Methods**

***Mike Elias****, Sandy Raimondo, Crystal Lilavois, Amanda Jarvis, James Justice and Kathryn Gallagher, U.S. Environmental Protection Agency*

Although numerous per- and polyfluoroalkyl substances (PFAS) are present in the aquatic environment, empirical toxicity data remain limited for a majority of these substances. Empirical toxicity datasets are relatively robust for perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) in freshwater environments, and US EPA recently derived draft aquatic life criteria for these substances using the traditional toxicity test-based derivation approach that EPA's Office of Water applies (1985 Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses). However, there remains the need to address potential risk to aquatic communities associated with the numerous other PFAS for which data are more limited. Accordingly, EPA applied a New Approach Methods (NAMs) tool to support the development of draft acute protective benchmarks for a selected group of data-limited PFAS. Specifically, Interspecies Correlation Estimation (ICE) model predictions were used in conjunction with available direct toxicity test data on the PFAS to support development of the underlying dataset for each substance. ICE-derived values and direct toxicity test data, once compiled, were then used to develop draft freshwater benchmark recommendations for each substance based on procedures consistent with those in the 1985 Guidelines. Deriving benchmarks using this approach is fundamentally consistent with the 1985 Guidelines "good science" clause and allows EPA to provide relevant information to states and tribes regarding protective values for aquatic life. Additionally, development of these PFAS benchmarks reflects goals in the EPA's PFAS Strategic Roadmap. Finally, these draft benchmarks are consistent with the Agency's intention to reduce the use of animal testing through the application of NAMs. This presentation provides both an overview of the process used to derive the benchmark values and the draft acute aquatic life benchmark values derived for the selected PFAS.

### 7.03.P-Th147 Development of the National Perfluorooctane Sulfonate (PFOS) Ambient Water Quality Criteria for Aquatic Life

*Amanda Jarvis, **James Justice**, Brian Schnitker, Mike Elias and Kathryn Gallagher, U.S. Environmental Protection Agency*

Perfluorooctane sulfonate (PFOS) is part of the broader group of per- and polyfluorinated substances (PFAS) which have unique chemical and physical properties, including thermal stability, water and oil repellency, and surfactant properties. PFOS was incorporated into a wide range of industrial and consumer products for decades until production was phased out by major U.S. manufacturers in 2002 with the exception of some small applications, such as chrome plating and aqueous film forming-foams. However, PFOS is highly persistent and remains present in aquatic ecosystems. Additionally, bioaccumulation of PFOS occurs through the aquatic food web and PFOS is frequently detected in organismal tissue. Thus, PFOS still poses a potential risk to aquatic life and aquatic-dependent wildlife. Acute and chronic effects to a wide diversity of aquatic and aquatic-dependent taxa have been reported in the current toxicity literature for PFOS. The Environmental Protection Agency is in the process of developing national PFOS Ambient Water Quality Criteria for aquatic life in accordance with the Agency's PFAS Strategic Road Map. Draft PFOS freshwater criteria and an estuarine/marine acute benchmark were released for public comment in April 2022. This presentation will provide an overview of the current development of national aquatic life criteria for PFOS with an emphasis on the evaluation of the current toxicity literature to support the derivation of water and tissue-based criteria. Once finalized, the PFOS criteria will be available for states and tribes to consider in adopting as their state water quality standards to ensure the protection of aquatic life from acute and chronic exposures of PFOS in freshwaters.

### 7.03.P-Th148 EthoCRED: A Framework to Guide Reporting and Evaluation of the Reliability and Relevance of Behavioural Ecotoxicity Studies

***Michael Bertram** and Tomas Brodin, Swedish University of Agricultural Sciences (SLU), Sweden*

Behavioural analysis has been garnering significant attention as a broad indicator of sub-lethal toxicity, and has secured a place as an important sub-discipline in aquatic ecotoxicology. One of the most notable characteristics of behavioural research, compared to other established approaches in sub-lethal ecotoxicology (e.g. reproductive and developmental bioassays), is the wide range of study designs being used and the diversity of endpoints considered. At the same time, environmental hazard and risk assessment, which underpins regulatory decisions to protect the environment from potentially harmful chemicals, often recommends that ecotoxicological data be produced following accepted and validated test guidelines. These guidelines typically do not address behavioral changes, meaning that these, often sensitive, effects are not represented in hazard and risk assessments. Here, we propose a new tool, the EthoCRED evaluation method, for assessing the reliability and relevance of behavioural ecotoxicity data, which considers the unique requirements and challenges encountered in this field. This method, and accompanying reporting recommendations, are designed to serve as an extension of the 'Criteria for Reporting and Evaluating Ecotoxicity Data (CRED)' project. As such, EthoCRED can both accommodate the wide array of experimental design approaches seen in behavioural ecotoxicology, and is able to be readily implemented into regulatory frameworks in different jurisdictions to allow better integration of knowledge gained from behavioural testing into environmental protection. Furthermore, through our reporting recommendations, we aim to improve the reporting of behavioral studies in the peer-reviewed literature, and thereby increase their usefulness in chemicals regulation.

### 7.03.P-Th149 Procedures to Derive Aquatic Life Benchmarks for Dichlorooctylisothiazolinone (DCOIT)

***Damani Parran**, Gary R. Long, Jalissa Nguyen and Andrew Patz, EHS Support LLC*

With the phase out of pentachlorophenol as a wood preservative, there is an industry-wide need to identify wood preservative alternatives. Dichlorooctylisothiazolinone (DCOIT) is an isothiazolinone, a class of heterocyclic compounds used as biocides and antifouling agents. DCOIT shows promise as a highly efficacious wood preservative; however, there are limited acceptable toxicological data available to derive aquatic life criteria to establish benchmark concentrations for use in discharge permits. As a result, uncertainty factors are required to derive Tier I or Tier II criteria for aquatic organisms under Great Lakes Water Quality Initiative (GLWQI) methodologies for developing aquatic life values, resulting in criteria that may be substantially lower than safe concentrations for aquatic life.

Recently, additional acceptable toxicity studies have been identified to improve the derivation of aquatic life criteria by increasing the minimum data requirements satisfied by available DCOIT aquatic toxicity dataset. Inclusion of these studies will reduce uncertainty factors, including the secondary acute factor (SAF) and final acute-to-chronic ratios (ACR), required in the Tier II calculations. We reviewed these additional studies to determine acceptability of the data and potential derivation of a more accurate aquatic life value. We will detail the findings of our review and assessment of the current data and provide recommendations on deriving more accurate and implementable aquatic life criteria for DCOIT under the GLWQI methodology.

## 7.03.V Deriving and Implementing Ecologically Relevant Water Quality Criteria and Guidelines

### 7.03.V-01 Water Quality Criteria and Ecological Risk Assessment of Typical Transition Metals in South Asia

***Ying Wang***[1]*, Tanjena Rume*[1]*, S.M. Didar-Ul Islam*[2]*, Jiangyue Wu*[3] *and Xiaomin Li*[1]*, (1) Beihang University, China, (2) Newcastle University, United Kingdom, (3) Ministry of Natural Resource of the People's Republic of China*

Transition metals water pollution in South Asia are more serious than other places due to the lack of technology and ability to treat sewage in this area. However, the transition metal pollution status and water environmental risk of the major rivers in South Asia are still unclear. The present study established species sensitivity distribution (SSD) models and then derived HC5s and WQC of six typical transition metals to South Asian aquatic organisms. The results showed that the order of acute WQC was Mn > Fe > Zn > Cd > Cu > Hg, and the sequence of chronic WQC was Fe > Cu > Cd. The risk assessment of these metals in the major rivers, including the Indus River, the Ganges River, the Brahmaputra River, the Meghna River, and the Bagmati River, were also carried out. It is found that the major rivers in South Asia are highly polluted via transition metals, and hence a large number of aquatic species are at toxicity risk. The results will help to understand the water environment and ecological risk of South Asia, and provide a scientific basis for strengthening the protection of water environment and revising the water quality standards.

## 7.04.P Leveraging Sustainability Initiatives to Provide FIFRA/ESA Mitigation Responses

### 7.04.P-Tu165 Broad Sustainability Goals Providing Benefit to Pollinators

*Chad Boeckman and **Michelle Blickley**, Corteva Agriscience*

Aside from traditional product specific environmental risk assessments, technology providers are increasingly engaged in broader sustainability efforts, the results of which are often not considered during the risk/benefit assessment conducted during product registration. One example of a broad sustainability goal is enhancing biodiversity on managed and/or natural lands. In an effort to operationalize the sustainability goal of enhancing biodiversity, Corteva Agriscience, working with established partner organizations, has implemented multiple programs focused on creating and/or improving pollinator habitat on various landscapes and educating youth and adults about pollinators, their role in the ecosystem, and establishment of pollinator habitat. This presentation will provide an overview of those programs and metrics collected to date on the number of acres implemented or enhanced. This overview should contribute to the symposium by providing relevant examples of actions taken under the umbrella of broad-based sustainability goals with the hope of furthering the conversation about potential opportunities and relevant challenges to using these types of efforts during product specific risk/benefit analyses.

**7.04.P-Tu166 Considering Co-Benefits and Co-Costs When Evaluating Pesticide Mitigation Measures**

***Annie Jean Krueger**, Leah Moore Duzy, Ashlea R. Frank and Bernalyn D. McGaughey, Compliance Services International (CSI)*

On January 11th, 2022 EPA Office of Pesticide Programs announced their new Endangered Species Policy stating their intent to evaluate the potential effects of any new conventional pesticide active ingredient on federally threatened or endangered (listed) species, and their designated critical habitats before granting registration. To aid their assessment, EPA has asked registrants to provide "upfront mitigations" to "minimize the effects of incidental take to listed species that could result from use of a pesticide." As pesticide registrants and EPA consider pesticide mitigation measures, it is important to consider what mitigation measures offer co-benefits for other environmental resource concerns and when mitigation measures may incur costs or conflict with other resource concerns. CSI will present a compilation of publicly available information on mitigation practices, co-benefits and limitations recognized by USDA, USFWS and peer-reviewed literature.

**7.04.P-Tu167 How Existing Initiatives Can Inform Feasible Pesticide Mitigations**

***Ashlea R. Frank**[1], Annie Jean Krueger[2] and Lydia Cox[3], (1) Compliance Services International (CSI), (2) University of Nebraska, (3) Nichino America*

In pesticide registration actions, the Environmental Protection Agency (EPA) must ensure that approved uses do not jeopardize the continued existence of species listed under the Endangered Species Act (ESA). Measures that restrict how or when the product is used can be added to pesticide labels to mitigate potential impacts of the pesticide on ESA-listed species. These measures can include areas where use of the pesticide must be avoided (avoidance) or measures that minimize the impact by limiting the degree or magnitude of the action or its implementation (minimization). In many cases, there are existing programs and initiatives already in place that effectively avoid or minimize exposure of pesticides to ESA-species. This presentation will provide examples of existing initiatives and how those can be considered in the pesticide/ESA assessment and labeling process to inform reasonable and feasible mitigations.

**7.04.P-Tu168 Species Vulnerability: A Data Centric Approach**

***Leah Moore Duzy**[1], Andrew Clawson[1], Ashlea R. Frank[1] and Lula Ghebremichael[2], (1) Compliance Services International (CSI), (2) Syngenta Crop Protection*

The terms "species vulnerability" and "vulnerable species" have been used interchangeably in a variety of forums, including registration decisions by the US Environmental Protection Agency (EPA) and US Fish and Wildlife Service's (FWS) Final Biological Opinion for malathion; however, the definitions and underlying data used to categorize species are not consistent, leading to unpredictable outcomes and perhaps oversight and exclusion of vulnerable species. With EPA's current focus on early mitigations, having a clear understanding of what characterizes a species as vulnerable, and consistent application of data being used to apprise those classifications are imperative to having informed discussions on appropriate mitigations. We have developed a quantitative approach for characterizing species vulnerability as part of an Avoidance, Minimization, and other Measures for Pesticides and Species (AMMPS) tool that incorporates similar data as EPA and FWS but is flexible to incorporate additional factors, such as existing mitigations. This presentation will overview the approaches used by EPA and FWS to characterize species vulnerability, highlight the benefits of using a quantitative approach to assessing vulnerability, and survey the data in the AMMPS tool for comprehensive assessment of species vulnerability.

**7.04.P-Tu169 Partnerships Lead to Exciting Progress in Species Conservation within the Agricultural Landscape**

***Timothy B. Fredricks**, Michael G. Dobbs and Aimee Hood, Bayer CropScience LLC*

As a major agricultural (Ag) innovation company, Bayer is aware of both the challenges to and potential for the Ag community to make meaningful contributions to sustainability and biodiversity targets, including endangered species, through farming and conservation initiatives. Our partnering with conservation groups, academic experts, farmers and government agencies across North America (NA) drives conservation within the Ag community. Through partnerships, Bayer can have a larger impact with on-the-ground habitat projects and more efficiently progress conservation. Along the way, relationships with diverse stakeholders have been established, trust and mutual respect have become the standard, which has enabled measurable improvements to our precious resources, flora, and fauna that are at the heart of all sustainability and biodiversity targets. Combining outreach/education, stewardship, along with habitat enhancement projects are often the most effective way to enhance biodiversity. Examples like monarch butterfly conservation efforts brought the plight of this iconic butterfly to the forefront of species conservation and research across NA, and Ag education and outreach programs that have helped communicate around pollinator, pesticide, and worker safety for growers/applicators. Benefits from monarch conservation go beyond one species, as good monarch habitat has broad conservation benefits by supporting many other insects, birds, and mammals while improving soil structure, water infiltration, and decreasing field run-off. Farming livelihoods and our safe and efficient food supply depend largely on the ability of farmers to produce more with less, bountiful harvests with minimal losses, higher yields on fewer acres, and regulatory certainty can help ensure these successes. How can these and similar proactive conservation and education efforts be built into the regulatory framework to help not only ensure adequate protections, but also that species have the necessary habitat to thrive. For example, off-labeling pesticide products in counties to protect endangered species could decrease exposure potential but will do nothing to help ensure high-quality habitat exists for species recovery. Recovery of endangered species is the ultimate goal. Through partnerships, with all stakeholders represented, Ag and regulatory organizations have the potential to chart a path that leads to continued food security, increased sustainability, and enhanced biodiversity.

**7.05.P Metals: Application of Models and Bioavailability Measures - Recent Developments**

**7.05.P-We188 Current activities towards updating U.S. Environmental Protection Agency's Aquatic Life Ambient Water Quality Criteria (AWQC) for metals**

***Christine Bergeron**, Kathryn Gallagher, Joseph R. Beaman and Luis Cruz, U.S. Environmental Protection Agency*

Aquatic Life Ambient Water Quality Criteria (AWQC) for toxic chemicals developed by the U.S. Environmental Protection Agency (EPA) are national recommendations to states and tribes of ambient water concentrations that will protect against adverse ecological effects to aquatic life resulting from exposure to a pollutant found in water. States, territories, and tribes may use the recommended criteria in developing water quality standards. EPA intends to systematically update AWQC for metals over time to reflect new toxicity studies and approaches for considering how water chemistry parameters (*e.g*., pH, dissolved organic carbon, and hardness) may affect metal bioavailability and subsequent toxicity to aquatic species. In March 2022, after undergoing independent, external peer review and revision, EPA published the report "*Development of an Overarching Bioavailability Modeling Approach to Support US EPA's Aquatic Life Water Quality Criteria for Metals"*. This report concludes Phase I of the Cooperative Research and Development Agreement (CRADA) that EPA entered with eight metals associations to develop a simplified, overarching modeling framework to predict the bioavailability of metals to support the development of updated metals Aquatic Life AWQC under the Clean Water Act. This report, developed in collaboration with the CRADA partners, provides a review of models that are available to predict the toxicity of metals to aquatic life considering factors that modify toxicity as a function of water chemistry. The document focuses on the performance of Biotic Ligand Models (BLM) and Multiple Linear Regression (MLR) bioavailability modeling approaches for aluminum, copper, lead, and nickel. Given the similarities in performance between the BLM and MLR approaches for several metals, EPA intends to use MLR models due to their relative simplicity, transparency, decreased number of input parameters and data collection requirements, and ease of use compared to the BLM. In Phase II, EPA is working with the metals associations to develop MLR bioavailability models for individual metals. After external peer-review of the models, EPA plans to develop updated, externally-peer reviewed Aquatic Life AWQC for metals to better support states, territories, and tribes. This presentation will summarize the findings and recommendations of the Phase I report and discuss the current activities under Phase II and criteria development.

**7.05.P-We189 Updates to the EPA Framework for Metals Risk Assessment**

***William J. Adams***[1] *and Emily Rogevich Garman*[2]*, (1) Red Cap Consulting, (2) NiPERA, Inc.*

In 2007 the US Environmental Protection Agency issued its "Framework for Metals Risk Assessment." The document is intended to provide technical guidance to risk assessors and regulators when performing human health and environmental risk assessments of metals. There have been several advances in the science related to metals risk assessment for the aquatic environment since 2007. This paper focuses on advances in assessing bioavailability in aquatic ecosystems, short- and long-term fate of metals in aquatic ecosystems and advances in risk assessment of metals in sediments. Notable advances have occurred in the development of bioavailability models for assessing toxicity as a function of water chemistry in freshwater ecosystems. BLM and MLR models now exist for most of the common mono- and di-valent metals. Species sensitivity distributions (SSDs) for many metals now exist making it possible for many jurisdictions to develop or update their quality criteria or guidelines. The understanding of the fate of metals in the environment has undergone significant scrutiny over the past 20 years or more. Transport and toxicity models have evolved including the Unit World Model (UWM) which allow for estimating concentrations of metals in various compartments as a function of loading and time. There has been significant focus on the transformation of metals in sediments to forms that are less bioavailable and to understanding conditions that result in re-solubilization or redistribution of metals in and from sediments. Methods for spiking sediments have advanced such that the resulting chemistry in the laboratory mimics that in natural systems. Sediment bioavailability models are emerging including models that allow for prediction of toxicity in sediments for copper and nickel. Biodynamic models have been developed for several organisms and many metals. The models allow for estimates of transport of metals from sediments to organisms via their diet as well as their water exposure. These advances expand the tool set available to risk assessors.

**7.05.P-We190 EPA GIS Tool Application for Displaying Water Chemistry and Freshwater Aquatic Life Criteria Values for Metals**

***Luis Cruz****, Mike Elias and Kathryn Gallagher, U.S. Environmental Protection Agency*

The United States Environmental Protection Agency (EPA) has developed 304(a) aquatic life metals criteria using bioavailability models to enable users to derive criteria that reflect site-specific water chemistry conditions. Water chemistry data inputs necessary to run metal bioavailability models may not be available or may be incomplete for some ambient waters. Thus, EPA is applying the United States Geological Survey's NWIS (National Water Information System) database in a Geographic Information System (GIS) map application to provide access to an interactive nationwide database to support states, tribes, and stakeholders with the derivation of bioavailability-based freshwater aquatic life criteria values when site-specific water chemistry data are not available. Only NWIS data from locations with at least 10 sampling events and with a full set of concurrently collected water chemistry parameters (Ca, Mg, Na, $SO_4$, Cl, K, Alkalinity, Temperature, Hardness, pH, DOC) were considered for use. In order for site data to be used, data had to be collected over at least three meteorological seasons with two or more sampled days per season. The resulting database consists of 43,718 records collected from 753 sites. Data were organized at three geographic levels for GIS display and subsequent calculations: a) by site, b) by level III ecoregion, and c) by Strahler stream order range within level III ecoregion. The 5$^{th}$, 10$^{th}$, 20$^{th}$, and 25$^{th}$ percentiles of the measured water chemistry parameters were calculated. The concurrently measured water chemistry data were also used as inputs into the 2007 Copper BLM and 2018 Aluminum MLR criteria calculators to derive acute (CMC) and chronic (CCC) criteria percentile values at the three geographic levels noted above. The GIS application presents data using two primary interfaces: 1) map layers with water chemistry parameters and calculated aquatic life criteria displayed at selected levels of organization, and 2) heat maps presenting the distribution of pH, DOC and water hardness in surface waters. The GIS maps can thus display water chemistry and aquatic life criteria percentile values by NWIS site, level III ecoregion, or stream order within ecoregions. EPA prefers stakeholders use current, site-specific, water chemistry data to develop criteria for metals but has developed this tool to fill in water chemistry data gaps when users are calculating bioavailability-based criteria values in data-limited situations.

**7.05.P-We191 Lessons learned from deriving arsenic human health water quality criteria in Idaho**

***Norka E. Paden**, Jason Pappani and Elizabeth Spelsberg, Idaho Department of Environmental Quality*

In September 2016, EPA disapproved Idaho's human health criteria for arsenic of 10 µg/L for consumption of fish only and consumption of fish and water. The Surface and Wastewater Division of the Department of Environmental Quality started a negotiated rule making process in 2018. One of the major tasks to develop the new arsenic human health criteria was to derive a bioaccumulation factor using Idaho-specific data. The current study provides arsenic accumulation monitoring in fish and water from 24 sites state-wide conducted in 2019. This analysis used EPA's recommended trophic-level bioaccumulation factor derivation approach. The analysis showed that, concentration of inorganic arsenic in the water column was not significantly related to the concentration of inorganic arsenic in fish tissue.

In the absence of a strong, significant, predictable relationship between concentrations of inorganic arsenic in water column and concentrations of inorganic arsenic in fish tissue, a more conservative approach for deriving water column criteria to be protective of designated uses was proposed. In addition, a fish tissue-based criterion that supersedes the water column element has been proposed. Because the fish tissue element supersedes the water column element, it is possible to use the direct measure of inorganic arsenic in fish tissue rather than rely on the conservative estimates of bioaccumulation when applying arsenic criteria.

**7.05.P-We192 A Case Study Demonstrating the Use of Multivariate Analyses and Metals Bioavailability Models to Understand Causes of Seasonal Sublethal Toxicity in Discharge from a Former Mine Pit**

***Shaun A. Roark**[1], Alexander Wilson-Fallon[1], Martin Powers[1], Jeremy Rigsby[2], Richard E. Lockwood[3] and Steven Brown[4], (1) Jacobs Engineering Group Inc., (2) FTN Associates, Ltd., (3) Ramboll, (4) The Dow Chemical Company*

Wilson Mine, a former vanadium mine in the Ouachita Mountains near Hot Springs, Arkansas (USA) has undergone extensive reclamation to mitigate risk of metals discharge into the surrounding watershed. Sediment capping in East Wilson Pond, a former mine pit, substantially reduced metal concentrations and associated toxicity at the pond outfall. However, seasonal sublethal toxicity to *Ceriodaphnia dubia* has been observed in association with stratification and reduced pH in the hypolimnion. Toxicity identification evaluations suggested that nickel, manganese, cobalt, and copper may have contributed to toxicity in the pond outfall, but that zinc was the likely primary cause of adverse effects. To better understand the causes of toxicity and inform risk management options, a multiple-lines-of-evidence approach was employed. Bioavailability modeling, including the Metals Bioavailability Assessment Tool and multiple regression models were used to estimate sample-specific metals probable no-effects concentrations (PNEC) and metals toxic units (TU) for multiple years of water chemistry data. Principal components analysis (PCA) was used to evaluate multivariate associations among metals, dissolved organic carbon, and pH over time. Correlations and concentration-response relationships for bioassay results and principal components (PC) associated with metals variation and metals toxic units, including the summed TU for zinc, nickel, and manganese, were evaluated. Results indicated that metals measured in site water are temporally intercorrelated, and the first PC, representing the variation in multiple metals concentrations at the site, was strongly correlated with *C. dubia* reproduction, explaining 80% of the variation in response. In log-logistic concentration-response models, the first PC was the best predictor of sublethal *C. dubia* response, followed by the sum TU for multiple metals, and then by the zinc TU. The integrated use of multiple lines of evidence, including PCA, bioavailability modeling, and concentration-response modeling, led to the robust conclusion that zinc was the primary cause of seasonal sublethal toxicity in the pond discharge, and other correlated metals, particularly manganese, may also contribute. These results were used to develop and apply modest additional management actions (ongoing) to further reduce risk from metals exposure and enable a final solution that is passive.

**7.05.P-We193 Using Multiple Lines of Evidence to Characterize Reductions in Metals Toxicity in a Groundwater-Surface Water System with Constructed Habitat Wetlands at a Former Vanadium Mine Site**

***Alexander Wilson-Fallon**[1], Shaun A. Roark[1], Martin Powers[1], Jeremy Rigsby[2], Richard E. Lockwood[3] and Steven Brown[4], (1) Jacobs Engineering Group Inc., (2) FTN Associates, Ltd., (3) Ramboll, (4) The Dow Chemical Company*

Indian Springs Creek (ISC) receives surface and subsurface water from Wilson Mine, a reclaimed former vanadium mine in the Ouachita Mountains near Hot Springs, Arkansas (USA). Biomonitoring indicated toxicity to *Ceriodaphnia dubia* downstream from the inflow of subsurface water and chemical analysis suggested manganese was likely the primary contributor. A multiple-lines-of-evidence approach was used to investigate spatial-temporal relationships between metals concentrations and *C. dubia* toxicity, and to evaluate the benefits of incorporating small, constructed wetlands (habitat wetlands) within ISC. Sampling locations for concurrent analysis of water chemistry and toxicity were established upstream and at several points downstream from the wetlands. The Metals Bioavailability Assessment Tool (MBAT) was used to estimate metals probable no-effects concentrations (PNECs) and metals toxic units (TUs). Principal components analysis (PCA) was used to evaluate associations among metals, dissolved organic carbon, and pH. Concentration-response relationships for *C. dubia* reproduction and metals concentrations, metal TUs, and principal components (PCs) associated with metals variation were also evaluated. The PCA indicated that metals (Mn, Ni, Co, Fe, Zn) were spatially and temporally intercorrelated, and *C. dubia* reproduction was negatively correlated with the first PC which explained 50% of the variation in reproduction. Bioavailability models indicated manganese TUs were consistently greater than one in samples where toxicity was observed, and concentration-response models confirmed that manganese was the best predictor of reduced reproduction (i.e., the primary cause of toxicity). Concurrent monitoring of water chemistry and *C. dubia* reproduction at stations along ISC demonstrated that the addition of small habitat wetlands reduced manganese concentrations more than 100-fold, and average reproduction improved from 7% of control upstream to 77% of control downstream of the wetlands during the 9 months studied. Further downstream from the wetlands, where additional mixing with surface water occurred and inconsistent sublethal effects were previously observed, manganese concentrations were reduced below the preliminary site-specific 20% effects concentration of 6,100 µg Mn/L, approximated the estimated PNEC (800 µg Mn/L), and toxicity appears to have been eliminated.

**7.06 Risk Communication: Strategies for Cross Communication Among Different Science Disciplines with Risk Issues**

**7.06.T-01 Sensationalized News and Emerging Contaminants: A Cautionary Tale from PFAS Applied to Microplastics**

***Rachel Zajac-Fay**, Tina Liu, Jean Zodrow and Jason M. Conder, Geosyntec Consultants, Inc.*

Risk communication is an essential aspect of risk assessment and toxicology yet it can be an overlooked tool. We live in the Digital Age where information is quickly and widely disseminated through all forms of media. With this abundance of information, scientists and science communicators specializing in emerging contaminants face a unique challenge in interpreting and communicating their findings. Published literature can easily be misconstrued and sensationalized for a news headline, which can cause unnecessary alarm from the public, create mistrust between the public and scientists, and cause confusion regarding actual toxicological information. We have seen this with PFAS, and we are seeing it with microplastics. Microplastics are one of the latest emerging contaminants that has come into the public eye; the quantity of peer reviewed literature has exploded in the past few years. With this new hot topic comes headlines such as, "You're eating a credit card worth of plastic each week." This presentation will explore the issues of sensationalizing toxicity of emerging contaminants, provide a case study using PFAS and lessons learned, discuss relevant microplastic headlines, and provide tools and best practices that scientists and science communicators can use when communicating emerging contaminants.

**7.06.T-02 Identifying and Understanding Residents' Risk Perceptions of Harmful Algal Blooms in Lake Wateree, South Carolina**

***Margaret A. Carson*** *and Geoffrey I. Scott, University of South Carolina*

Background: Harmful algal blooms (HABs), such as cyanobacteria algal mats, have been detected in Lake Wateree, South Carolina as early as 2012. Toxicity tests indicate that the toxin found in these algal blooms, Lyngbya wollei toxins, has the potential to be harmful to humans, pets and aquatic species. This study aimed to identify and understand residents' perceptions of risk of the HABs in efforts to create communication materials for the purposes of informing residents of pertinent water quality, public health, and behavior modification information in support of science-based decision making.

Methods: An ethnographic, qualitative study using, in-depth, semi-structured, interviews was conducted with Lake Wateree, South Carolina residents. Interview participants had to meet inclusion criteria to participate in this study. Interviews were recorded and transcribed verbatim using a professional transcription service. Data was analyzed with a thematic analysis technique for both a-priori and emergent themes. NVivo, a qualitative data analysis software, was used to organize data and assist in data analysis. Participants were asked questions related to perceptions, attitudes, knowledge of harmful algal blooms in Lake Wateree. Additionally participants were asked ideal ways to receive communications on water quality, have they ever experienced health issues related to lake use, and when and what type of contact recreation participants utilized the lake for.

Results: The results suggest that the majority of participants were aware of the presence of HABs. Majority of self-reported HAB knowledge levels were high. Resident presented negative attitudes toward the presence of HABs in the lake. Key emerging codes were community wants, health concerns, perceptions of the algae and resident proposed algae solutions.

Conclusion: We conclude that the presence of the algal mats has altered how residents use Lake Wateree to some extent. It is necessary to have multiple channels for effective communication. We propose a centralized information portal that connects residents, community advocacy groups, companies and utilities that utilize the lake, the local research university, and the electric power holding company, who manages the lake with sound science-based information to inform decision making.

**7.06.T-04 Implementation of Risk Communication Best Practices at EPA: A Case Study on Engagement**

***Madeline Beal****, U.S. Environmental Protection Agency*

EPA's mission is to protect human and environmental health. We cannot achieve this mission without communicating about risk to a wide variety of audiences. Often, EPA must communicate about current health risks in a context where the solutions to that risk will be both incomplete and take a long time to achieve. Building trust in this context is extremely challenging. This is no where more true than when engaging directly with communities that face a disproportionate share of pollution, have fewer resources to advocate for themselves, and often have long-standing and well-reasoned distrust of government. The author will present a case study related to community engagement on cancer risk related to air toxics. This case study will include both an internal to EPA and external focus on the challenges and lessons learned from a process of implementing validated risk communication science principles into practice on an issue that is national in scope but local in impact.

Many of the lessons learned on this project are focused on internal organizational challenges rather than questions over what is the right approach to use (though aspects of the approach will be discussed and the author will explain EPA's new SALT Framework for risk communication.

There is focus within the risk communication field on messaging, tactics, platforms, and approaches. There is less focus on how organizational issues can stand in the way of actually implementing those messages, tactics, platforms, and approaches. Internal risk communication is often as important if not more so than external risk communication and organizational structure must allow risk communication to be prioritized. This often requires communicating across not just disciplines of science, but across policy, enforcement, and communications disciplines as well.

**7.06.T-05 Risk Communication to Reduce the Public Health Burden of Wildland Fire Smoke**

***Mary Clare Hano**, U.S. Environmental Protection Agency*

In this presentation, we will share current research on innovative actions to reduce the public health burden of wildfire smoke through health risk communication with stakeholders from a broad range of backgrounds.

This research leverages an applied, community-engaged design, and is led collaboratively by social scientists with the U.S. Environmental Protection Agency (EPA) Office of Research and Development and local public health leaders in Butte-Silver Bow, MT and Garfield County, CO. In both communities, local leaders convened multiorganizational teams to engage in learning about the public health issue of wildland fire smoke exposure and designing a communication plan that is tailored to the needs of their community. In the communication plan design process, team members leveraged EPA's SALT Framework, which emphasizes the importance of Strategy, Action, Learning, and Tools in risk communication initiatives. Communication strategies for specific audiences will be implemented in the 2022 wildland fire season and evaluated to identify opportunities for refinement and improvements.

The increasing occurrence of wildland fire smoke events emphasizes the need to communicate the actions that individuals, organizations, and communities can take to reduce exposure and mitigate the associated adverse public health outcomes. The local public health smoke communication plans are designed to share public health messaging with those stakeholders before, during, and after smoke events to support community members in taking action to reduce their exposure to wildland fire smoke.

As scientists who study human health and the environment, we all have a responsibility to learn and practice effective risk communication with different audiences who might benefit from our research, even if our research does not use an applied, community-engaged study design. Learning effective strategies to communicate about environmental health research is an opportunity to increase the impact of your work and practice a growth mindset to foster personal development. Focus group, observational, and community assessment data will be shared and used highlight a process that attendees may use as a guide for identifying who might benefit from learning about their work and how to integrate communication as a central component of our environmental health research. Disclaimer: This abstract does not reflect the policy of the U.S. EPA.

**7.06.T-06 Science Communication through social media campaigns and open virtual events**

***Corinna Singleman** and Nathalia Holtzman, Queens College*

Science communication, from discussing risk management to describing novel data to explaining science in the classroom, is vital to the progress of not only scientific fields, but also to society's understanding of the value of science. Engaging the public and the communities we serve with real scientists and connecting them in unique ways is just as important as submitting a well written document to a journal. The HSI-STEM Bridges Across Eastern Queens project in Queens, NY, aims primarily to enhance STEM education in introductory science courses at Queens College and Queensborough Community College. Since the project's inception, it has become clear that improving content communication within courses is not enough; broader scale communication with the college community and the broader community is needed. To this end, the project coordinators used social media as a tool to disseminate information directly through posting campaigns and as a forum to invite participation in live events.

Using social media, we worked to share the faces and stories of scientists to show the increasing diversity of scientists during celebratory/commemorative months (i.e. Hispanic Heritage Month, Pride Month, etc.) including photos, quotations, and brief information about each scientist. While these monthly social media campaigns increased our social media followers each month, we also wanted more live engagement. We held panel discussions with some of the featured members of each community and hosted seminars with invited speakers. The panels and seminars not only served to share the stories of the speakers, but this strategy also allowed for live interaction between scientists and the public fostering in-depth and thought provoking discussions.

These activities are useful in engaging members from all stakeholders. While our primary focus in utilizing social media was to share information with the lay public, our website (which all social media platforms highlighted and linked to) includes materials for other scientists, government stakeholders, and those who are interested in delving deeper into our project plans and outcomes. The website includes links to the project design, literature review, and our peer-reviewed published papers from the project. This interplay between interactive social media, formal web design and scientific materials provides a novel approach for using well established tools to communicate science in new ways.

**7.06.T-07 Public Participation and Lessons Learned from a Successful Residential Remediation**
***Amy Goldberg Day***, *Arcadis U.S., Inc.*
In 2015, the city of Whittier California was performing geotechnical sampling in preparation for a bike path installation along a railroad right of way. The contractors discovered a large amount of blue-green material slightly below the surface between the active rail corridor and residential backyard fences. This material was analyzed and reported to contain arsenic, lead, copper, and zinc at concentrations above hazardous waste classification. Because the discovery was on the railroad property, the railroad retained an environmental consultant to evaluate the soil conditions in the surrounding area and to identify potential sources. The California Department of Toxic Substances Control (DTSC) was notified and became the oversite agency.

Soil sampling and subsequent data evaluation was performed for site characterization purposes. The soil analytical results indicated that several adjacent residential properties were affected with high metals concentrations. Even though the source of the blue-green material was unknown, DTSC attributed its presence to railroad operations. In total, seven residential properties were identified with elevated metals in backyard soils. Cleanup goals were established, and the railroad representatives worked closely with the residents and DTSC to remediate the backyards.

This project was more than just remediating backyards. It involved working with the regulatory agency, and continual communication with the residents through frequent status update emails and mailers. In addition, periodic neighborhood meetings were held to discuss remediation progress and respond to community questions and concerns. The rail company took responsibility and was attentive and respective of the residents' concerns, which contributed to the residents feeling involved in the overall process. The public participation empowered the community and was an important part of the total remediation.

This presentation will discuss the lessons learned and what key elements of the public participation were paramount to the project's success.

**7.06.T-08 Strategies for Cross Communication Among Different Science Disciplines - A Panel Discussion**
***Shawn Sager***[1], *Rachel Zajac-Fay*[2], *Madeline Beal*[3] *and Jacquelyn Clarkson*[4], *(1) Arcadis U.S., Inc., (2) Geosyntec Consultants, Inc., (3) U.S. Environmental Protection Agency, (4) Louisiana Department of Natural Resources*
Risk communication is the interaction between environmental risk assessment scientists, managers, policy

makers and the interested public. With information readily available from the internet, mass media, and "24/7" journalism, communicators need to be cautious and thorough when interpreting and communicating information to stakeholders. Many environmental risk assessors, managers, and policy makers are uncomfortable in the role of risk communicator, and they have often overlooked the importance of being able to communicate effectively with the interested public. Therefore, risk communication remains one of the most underused tools for environmental management. The objective of this session will be to identify successful risk communication strategies as tools for use by science and risk communicators, which can include the effective use of communication programs.

This panel discussion will engage the audience and all the speakers for the session in a two-way conversation designed to highlight effective risk communication strategies as tools for use by science and risk communicators. The co-chairs for the session will moderate the discussion. Each presenter will provide a single summary slide to the moderators, who will review and collate the information. The combined summary will be used as a starting point for discussion and for drawing questions and comments from the audience. The moderators will also create a list of questions and comments for the panel, to ensure a robust and lively discussion is presented. If needed, the panel can respond to any questions that were not addressed during the individual presentations due to time constraints. Through our presentations and panel discussion, we will highlight ways to make science more accessible and useful to stakeholders. Ultimately this will promote more useful science, and greater public awareness of the importance of science.

**7.06.V Risk Communication: Strategies for Cross Communication Among Different Science Disciplines with Risk Issues**

**7.06.V-01 Risk Communication - The Power of One-On-One Communication**
***Megan Hamilton*** *and Sarah Jonker, Arcadis U.S., Inc.*
Investigating and remediating environmental releases often necessitates communication with adjacent residential and commercial property owners. This is especially true when investigating the vapor intrusion (VI) pathway, as VI sampling methods require members of the community to allow environmental professionals into their homes and workspaces. The VI evaluation process can include activities, such as: inspections of all inside and outside areas of a property; interviews with occupants to discuss everyday behaviors, habits, and hobbies; evaluation of chemical and product usage; visible placement of sampling apparatus; and even drilling of holes in building foundations. These intrusive methods can give rise to many different concerns for the property owner and can create feelings of fear, anger, distrust, and result in very high emotional situations. Risk communication in these scenarios becomes key and implementing the right tool at the right time can help to alleviate some of the associated concerns.

Many tools are available to aid in the risk communication process. While large-scale mailings and public meetings can assist in disseminating information to many people in a short timeframe, and may be necessary for some projects, the value of one-on-one communication should not be underestimated. One-on-one communication can help build trust and collaboration, especially in such personal situations as investigations inside someone's home. Consistent one-on-one communication can also prevent wide-spread panic and the need for large public meetings, which can draw unwanted media attention and create a sense of "us vs. them" mentality. This presentation will present several case studies where one-on-one communication proved successful at alleviating community members' concerns and promoting collaboration, while moving projects forward towards the closure process. The presentation will also provide examples of how to incorporate on-on-one communication into larger public discussions, in the event a public gathering becomes a necessary part of the risk communication process.

## 7.07 Role of Scientific Societies: Gate Keepers of Current Scientific Norms or Platforms for Presentation of Diverse Ideas?

### 7.07.T-01 Science Under Attack: Why We Should All Care About Censorship In Science

***Patrick D. Guiney***[1], *Tim Canfield*[2] *and William L. Goodfellow*[3], *(1) University of Wisconsin, Madison, (2) U.S. Environmental Protection Agency, (3) Exponent*

Science is under attack. Ecotoxicology and environmental chemistry are not exempt from this pressure. Peer-reviewed papers are being retracted or labeled as "fake news." Often this pressure seems to be generated by social media networks without adequate facts or justification. Scientists are blocked from speaking to the media, research funding is reduced, publication and presentation opportunities are denied, and policy decisions are being made that can undermine our ability to further explore and protect human health and the environment. These decisions may be in direct conflict with the scientific evidence. What is even more disturbing is that some people and even some scientists seem to think that this is proper behavior. They argue that the peer-reviewed science literature is flawed, that scientists do a poor job of self-correcting our mistakes, and these behaviors are needed steps toward progress. Censorship of science does not correct the problem but rather introduces new ones. Censorship can be deliberate as mentioned above and in the case of purposely suppressing data. Just as important however, censorship can occur indirectly through the limitation of a scientist's freedoms to discover and express hypotheses. Scientists who do research or speak out against scientific norms can be seen as a threat by vested interests and may come under attack or cancelation (i.e., ostracization by colleagues/blacklisting, harassment, negative social media campaigns, disapproval from superiors, threats, reprimands, denial of grants or other funding, promotion or job denial, punitive transfers, legal actions, or dismissal). Stifling lines of research by establishing research priorities that support funding of specific "niche" research, often performed by a cloistered group of researchers bolstering the current scientific norms. Vast amounts of money are often available for certain areas, which in turn encourages more scientists to decide to work in those areas, while neglecting other equally important research areas. We offer suggestions on how to combat censorship in science from improving our scientific communications, hurdles to improve publication success, encouraging new data sharing initiatives like those from NIH which advance open science, and tolerating ideas that make us uncomfortable or even angry because some of them might be right. We all should care about censorship because it threatens open discussion of science and policy.

### 7.07.T-02 The critically important role of scientific societies: perspectives from academia to government service to chemical industry scientist

***Laura McConnell***, *Bayer CropScience LLC*

Take a moment to imagine science without independent and robust scientific societies. The pace of scientific advancement around the world would likely be much slower. Scientific societies are important collision spaces for developing new ideas, are platforms for building collaborations, and they support the development of early career scientists. Scientific societies which maintain an ethical, open and transparent approach to their operations are critically important to allow for advancement in science. Well-managed scientific societies are also places where honest, respectful debate can occur on controversial topics. Over the last thirty years, I have progressed from earning a Ph.D. in academia, to a twenty-year career as a research scientist in federal service, and now to regulatory scientific affairs in chemical industry. In each role, scientific societies like SETAC have influenced the direction of my research program and have greatly enhanced my professional development. SETAC, with its explicitly stated tripartite model, has established a platform where scientists from different sectors are encouraged to engage and collaborate on a more equal basis. I will review how my relationship with SETAC has evolved as moving from government researcher to industry scientist, and I will share some observations of different science societies in comparison to SETAC.

### 7.07.T-03 The Pitfalls of Fighting Manufactured Doubt

***Annegaaike Leopold*** *and Gunilla Oberg, University of British Columbia, Canada*

Manufactured doubt, which aims at delaying policy action, has been observed in many debates around topics of human health and environmental concern. It is essential to fight manufactured doubt, especially in the current era of alternative facts and disinformation. In the eagerness to do so, however, there is a risk of stifling the essential exchange of differing scientific views thereby manufacturing a consensus in complex areas of societal concern where a healthy scientific debate is crucial for rigorously informed and wise policymaking.

The need to consider different scientific perspectives is all the more important, because scientifically sound reasoning can support different societal interests, as well as political and entrepreneurial decisions. To add to the complexity of scientific perspectives, there is a growing recognition in many countries, including Canada and the US, that many of our present-day systems rely heavily on a narrow definition of what is relevant and valuable knowledge and expertise. These insights have led to increasing calls for interdisciplinary, trans-academic and cross-cultural competency, which has proven to be more challenging than anticipated. The authors will build on their 30+ years of experience in respectively, regulatory environmental science and the evaluation of endocrine disrupting substances, and in leading and participating in sustainability research in areas of contemporary societal concern. They will examine the stumbling blocks to finding more equitable and robust ways of managing chemicals, including the ways in which disciplinary and cultural arrogance hinder the development of common ground and respect for other ways of knowing. They will discuss how a systematic fostering of humility, reflection and true dialogue can be used as a means of increasing awareness of how differences in thought styles, in writing and oral expression styles can impact individual and societal perceptions of environmentally sound solutions that have rigor, reliability and relevance.

**7.07.T-04 The Demon-Haunted World, The War on Science, Bending Science and the attack on scientific inquiry from the outside and within.**

***Wayne G. Landis**, Western Washington University*

Carl Sagan's *The Demon-Haunted World* (1998), Otto's *War on Science* (2016) and the classic *Bending Science* by McGarity and Wagner (2008) have documented the attacks on the scientific process by a number of outside forces. Rohr's The Atrazine Saga and its Importance to the Future of Toxicology, Science, and Environmental and Human Health (ET&C, 2021) describes the suppression of information regarding the estrogenic effects of atrazine by a number of organizations and individuals. There are many other examples to be found in the History of Science literature regarding the attacks on science regarding the effects of smoking, the existence of anthropogenic climate change, and more recently the virulence of and treatment for COVID 19. When I have asked questions at local, national and international SETAC meetings the appears to be a general ignorance of this literature and its effects on science and the making of environmental policy. As an editor and committee member for publications from ASTM, SETAC and the Society of Risk Analysis I may be particularly sensitized to the literature and its impacts. In this presentation I am delineating several case studies on how the war on science is managed, how it can be found in the business, academic and governmental sectors, how the pressure to publish and to fund laboratories that generate overhead plays a part, and the current backlash on expertise exacerbates the problems. Scientific publishing is also a huge profit-making enterprise. To finish I am outlining the steps that journals and publishers can take to preserve the integrity of the science while still promoting the intense technical scrutiny necessary to determine the structure of nature and policy decisions based on facts.

**7.07.T-05 SETAC Should Provide a Forum Where Diverse Ideas are Welcomed and Scrutinized**

***Katherine K. Coady**, Bayer AG - Crop Science Division*

SETAC's mission is: "to support and facilitate the development of principles and practices for the protection, enhancement and management of sustainable environmental quality and ecosystem integrity" and its vision is: "Environmental Quality Through Science®". To realize this mission and vision of protecting the environment, it is imperative that new ideas and approaches from diverse perspectives are encouraged within the Society. Likewise, new and diverse ideas and approaches should be met with scientific scrutiny within the Society to ensure that science leads the way in decision-making. SETAC already embraces a multidisciplinary approach to its proceedings, which can encourage diverse perspectives, but, if misapplied, could drown out divergent ideas without due consideration. This presentation will discuss attributes that should be considered within scientific societies to ensure that diverse ideas and perspectives are welcomed, but also held up to scientific scrutiny. This can indeed be a challenging balancing act! The attributes of humility and open-mindedness will be discussed as well as approaches to ensure scientific rigor, such as systematic review and weight of evidence evaluations. Ultimately, a strength of SETAC is its inclusion of multidisciplinary perspectives, which should not be used to stifle more divergent ideas or approaches. Rather, ideally, the role of a scientific society should be to enhance scientific discourse in a professional atmosphere.

**7.07.T-06 Your Censorship versus Mine; An Attempt to Falsify My Own Arguments Against Censorship**
***Christopher Borgert****, Applied Pharmacology & Toxicology, Inc.*
This session is focused on what is perceived to be an increasing and detrimental use of censorship to prevent the release of data, to silence interpretations, and to excommunicate scientists that contradict or undermine the views of those in positions of political or regulatory authority. If we are to consider this "hypothesis" scientifically, we must test it rigorously through falsification, at least logically and empirically of not experimentally. This talk will focus on the latter assertion of the hypothesis, that censorship in science is detrimental. Although often argued as an illegal or unethical infringement of the right to free speech, we can readily observe that censorship is a societal norm rather than an exception. Individuals, in fact, self-censor to avoid social embarrassment, ostracism, or even legal penalties, and we expect civil authorities to enforce censorship in many circumstances. We don't cry "fire" in a crowded theatre, for good reason. Censorship has tangible benefits in avoiding danger and preventing harm, but censorship also protects the dignity of individuals and promotes fairness within advanced societies, most of which have laws against against slander and libel, which are also forms of censorship. Within science, another form of censorship – peer-review – is not only the rule, but a practice about which we boast and in which we feel obligated to promote and to participate. Would anyone argue that peer-review, or at least the original intent of peer-review, is not a benefit to science? Yet, the practice of peer-review has been criticized not only for ineffectiveness but for unfairness, and worse. From these observations, we might conclude that censorship is not only ubiquitous but complex. Acknowledging the depth and breadth of that complexity may point to the crux of the issue: under what conditions and for what purposes is censorship beneficial and therefore appropriate, versus detrimental and inappropriate, or worse? Under what premises and under what rules should censorship be governed, and how can we, as a scientific society, partner with other scientific societies to ensure the most open exchange of ideas, data, and interpretations without compromising scientific standards of rigor and integrity? Finally, what remedies can be implemented to discourage the misuse of censorship at all levels of scientific discourse and application, and what penalties should extend to those who breach this code of conduct?

**7.07.T-07 Panel Discussion 1 of 2: Role of Scientific Societies**
***Tim Canfield***[1]*, William L. Goodfellow*[2] *and Patrick D. Guiney*[3]*, (1) U.S. Environmental Protection Agency, (2) Exponent, (3) University of Wisconsin, Madison*
Despite on-going efforts both inside and outside of SETAC, we believe that we have a crisis in science. From the general public to regulators and legislators, people continue to have distrust in scientists and by extension science itself. This year's session focuses on issues related to censorship in science which we believe is becoming increasingly prevalent. Plans for the session are 6 individual presentations followed by approximately 30 minutes of a panel discussion. The panel will address questions from the session chairs as well as questions from the audience (both in person and virtual). Over the last several annual meetings we have been excited by the interest and dialog created by this panel format. Session Chairs ask that open questions based on the published abstract information for each talk be sent prior to the session to wgoodfellow@exponent.com.

**7.07.T-08 Panel Discussion 2 of 2: Role of Scientific Societies**
***Tim Canfield***[1]*, William L. Goodfellow*[2] *and Patrick D. Guiney*[3]*, (1) U.S. Environmental Protection Agency, (2)*

*Exponent, (3) University of Wisconsin, Madison*
Despite on-going efforts both inside and outside of SETAC, we believe that we have a crisis in science. From the general public to regulators and legislators, people continue to have distrust in scientists and by extension science itself. This year's session focuses on issues related to censorship in science which we believe is becoming increasingly prevalent. Plans for the session are 6 individual presentations followed by approximately 30 minutes of a panel discussion. The panel will address questions from the session chairs as well as questions from the audience (both in person and virtual). Over the last several annual meetings we have been excited by the interest and dialog created by this panel format. Session Chairs ask that open questions based on the published abstract information for each talk be sent prior to the session to wgoodfellow@exponent.com.

**7.08.P Strengthening the Interface Between Science and Policy for Reducing Pollution from Chemicals and Waste**

**7.08.P-Tu172 Improving Cooperation Across Numerous Interfaces of Action and Information Sharing to Enhance Monitoring of POPs in Air To Support Domestic and International Chemicals Management**
***Tom Harner*[1]*, Jacob Mastin*[1]*, Amandeep Saini*[1]*, Lauren South*[1]*, Jasmin Schuster*[1] *and Ramon Guardans*[2]*, (1) Environment and Climate Change Canada, (2) MITECO, Spain*
The 3rd global monitoring report of the Stockholm Convention on POPs is nearly finalized and presents time trend information for core media which include air, human tissues, and water (for the listed PFASs). These data are used to inform Effectiveness Evaluation (Article 16) of the Convention regarding the impact of regulatory measures to protect human health and the environment from the harmful effects of POPs. In the case of air monitoring data included in GMP, good progress has been made over the past decade but large gaps in reporting exist, which limits the ability of the GMP to inform the Effectiveness Evaluation process and the Conference of the Parties (global community). Largest data gaps exist in developing countries and regions. A review exercise was initiated to assess the current situation with respect to air monitoring capacity for POPs, and to explore how this capacity could be better integrated into the work of the GMP as well as national needs. These enhancements will help to address the growing challenges associated with an ever-increasing list of POPs - which now includes 30 chemicals/classes, some of which are complex mixtures, and analytically very challenging. Pragmatic and cost-efficient solutions were identified, which start with better engagement and inclusiveness across the science-policy interface; better awareness of co-benefits across the policy-policy interface; and improved outreach to the public (public-science and policy-science interfaces). These approaches are consistent with, and inspired by, recent messaging from the scientific community for the need to enhance international cooperation, clarity and scientific rigour in support of science-policy interactions to address pollution and waste in increasingly effective strategies. Finally, the adoption of passive air sampling and improved partnerships with existing programs and key players (across all interfaces) is proposed as a strategy to address the growing demands on monitoring. It is unrealistic and unfair to expect all countries and regions to meet the challenges on their own. It has been shown that long term cooperation in distributed networks sharing methods, labs and sample banks can be very effective in acquiring valuable knowledge on changes over time and building professional competence in all regions.

**7.08.P-Tu173 Water Solubility and Kow of Modified Rosin Ester for Regulatory Registration**
***Guangshu Zhai*** *and Patricia Tcaciuc, 3M Company*
The water solubility and the n-octanol/water partition coefficient (Kow) have been measurements prioritized by regulators for the global chemical regulatory registration, such as EU Registration, Evaluation, Authorization and Restriction of Chemicals (REACH). There are few studies for complex mixture in the literatures and current OECD methods. In this project, the water solubility and Kow of a complex mixture (Modified Rosin Ester) were studied for REACH registration.

Determination of water solubility and Kow of Modified Rosin Ester was very challenging because it was a complex mixture without a well-defined composition and with very low water solubility. Three primary components, including diethylene glycol diester (DGD), glycerol diester (GDE) and glycerol triester (GTE) exceeded 10% and were monitored. Furthermore, DGD, GDE and GTE were also mixtures, with each containing multiple compounds originally derived from the various natural rosin acids, such as abietic acid and neoabietic acid. Its exact composition of these acids was not known.

All possible molecular weights of the rosin acid and ester combinations were thus calculated for the LC-MS/MS method. Since isomers may have different fragmentation patterns, a novel LC/MS/MS method was developed using the same masses in the mass transition Q1/Q3, which greatly reflected the primary components and escaped the issues of unknown composition ratios and the unknown ionization efficiency and product ions. The newly developed method was successfully applied to the study of the part-per-billion (ppb) level water solubilities of Modified Rosin Ester.

The low water solubility of Modified Rosin Ester posed more challenges for the Kow study which would put it outside the applicability domain of a shake flask Kow method. The HPLC method based on the retention times was adopted instead, as described in OECD Guideline 117. HPLC with UV detector was used to determine the retention times. However, Modified Rosin Ester could not be eluted from the column using the OECD 117 recommended method with mobile phase of 75%/25% (v/v) methanol/water within 60 min (suggesting that the Kow was very large). Therefore, it was concluded that the log Kow of the Rosin ester was >5.7 (which satisfied the data requirement for the REACH registration), but in order to confirm Modified Rosin Ester present in the Kow sample, the 95%/ 5% (v/v) methanol/water gradient was used to elute them from the column to confirm with LC-UV-MS/MS method.

**7.08.P-Tu174 Airborne Polychlorinated Biphenyl Remediation in Schools: Room-to-Room Differences, Source Emissions, and Community Engagement**

***Moala K. Keshei**, Jacob Jahnke, Rachel Marek, Craig Just and Keri Hornbuckle, University of Iowa*

Inhaled exposure to polychlorinated biphenyls (PCBs), a human-made group of 209 organic chemical pollutants, has been linked to cancer, hormone disfunction, and cognitive learning disorders regardless of age or sex. There is evidence that historic and modern PCBs still exist in the environment today, specifically within schools and offices. To reduce cost and improve efficiency of airborne PCB remediation we hypothesize that we must

1. Examine the differences in airborne PCB concentration and congener distribution from room-to-room in a single school.
2. Identify the relationship between novel sources of airborne PCB emissions indoors and room concentrations.
3. Understand what forms of science communication help inform community stakeholders on the issue of airborne PCB contamination in schools.

By using polyurethane foam passive air samplers, we found that multiple rooms within the same school building have different airborne PCB concentrations (between 1.5 and 35 ng $m^{-3}$) and different congener distributions (cosine theta > 0.90). We used positive matrix factorization to confirm these observations were caused by different PCB emission sources from room to room. To further investigate differences in source emissions, we sampled airborne PCB emissions directly, using polyurethane foam passive emissions samplers, before and after hexane wiping from carpet, wood panel walling, and hallway tile from multiple rooms in an office building. We found that all these materials emitted airborne PCBs before and after the removal of surface PCBs. The difference before and after wiping for carpet (n=6) was 6187 ± 2363 ng $m^{-2}d^{-1}$, wood panel (n=6) was 3832 ± 1603 ng $m^{-2}d^{-1}$, and tile (n=3) was 11,841 ± 5442 ng $m^{-2}d^{-1}$. Surface PCB removal does significantly reduce emissions across all materials measured (p-value = $1.2 \times 10^{-4}$) meaning that a removable surface layer of PCBs indoors is a distinct emissions source. Additionally, most, but not all 209 PCB congeners were significantly reduced by hexane wiping (59%). We held conversations with community stakeholders such as school district superintendents, grounds staff, and appointed government officials. Stakeholders use multiple forms of science communication to inform their decisions on airborne PCB remediation in schools from rigorous peer-reviewed journal articles to easily digestible, short presentations. We participated in several types of community-centered report back such as press releases and specialized lectures.

**7.08.P-Tu177 Managing the Environmental Risks of Medicines via Multi-Stakeholder Partnerships**
*Cristiana Cannata[1], Caroline Moermond[2], **Joan G. Tell**[3], Rhys Whomsley[4] and Peter Wilson[5], (1) Radboud University, Nijmegen, Netherlands, (2) RIVM, (3) Merck & Co., Inc., (4) European Medicines Agency, Netherlands, (5) Sanofi, France*
PREMIER (Prioritisation and Risk Evaluation of Medicines in the EnviRonment) is a 6-year research project driven by the EU Innovative Medicines Initiative (IMI) designed to bring regulators, academia and industry together to tackle the issue of pharmaceuticals in the environment (PIE). PREMIER brings together a world-leading multidisciplinary consortium of 25 public and private partners committed to minimizing the risks of PIE through collaboration. Critical deliverables include new *in vitro* and *in silico* tools to identify and predict potential environmental impacts associated with the use of human medicines, a framework to prioritise the environmental assessment of existing medicines based on risk, and new environmental fate and effects data on key pharmaceuticals based on intelligent design principles to reduce duplication and minimize animal testing. Options for greener drug design that are used to examine the feasibility of incorporating this concept in drug discovery and development are also being developed in concert with experts from biopharmaceutical research & development. We will create a public and easily accessible database collating physico-chemical and environmental properties (ecotoxicity, e-fate) as well as relevant metadata for human medicines. Importantly, a guiding system to support regulators, water managers and other stakeholders in identifying and managing any potential environmental risks associated with the patient use of medicines will be developed. An update of the project as it completes Year 2 will be provided.

**7.08.P-Tu178 Methodology to assess the environmental implications of agricultural systems to inform sustainability improvements by taking into account environmental costs and benefits**
*Laura Green[1], Elise Webb[1], Elizabeth Johnson[1], Gregor Morgan[1], Sarah Wynn[1] and **Christian Bogen**[2], (1) ADAS Ltd., United Kingdom, (2) Bayer AG - Crop Science Division, Germany*
With the United Nations Food Systems Summit and the European Green Deal there are currently various policy and regulatory initiatives to transition agriculture with the aim to become more sustainable. Nonetheless, in many of those approaches a detailed perspective on agronomic challenges and the consideration of the farmers opportunities to handle those appears largely absent. However, to improve sustainability of farming it is important to understand the environmental costs and the benefits of the various tools and methods that are applied within diverse cropping systems.

Based on the available peer-reviewed scientific literature, we developed a methodology that used the environmental categories that are usually addressed within environmental risk assessments for plant protection products, and applied it to various other, non-chemical tools that are commonly part of agriculture.

In a second phase of the work, we explored the scientific literature about the environmental implications of the production systems where those tools and methods are applied. We took the example of conservation agriculture with a focus on wheat production to explore the environmental benefits and limitations of that specific system.

The results of the first work phase show a nuanced picture of the environmental implications related to direct impacts, indirect impacts, their selectivity and potential data gaps when it comes to the different tools and impact categories. The currently investigated tools and methods comprise ploughing, crop rotation and the use of a combine harvester.

For conservation agriculture a multitude of environmental benefits were found in the investigated peer-reviewed literature, although the system may also present some limitations, particularly for temperate climates. The decision to implement conservation agriculture would therefore require consideration of a wide range of environmental and socioeconomic factors.

The two-phase assessment of the different tools and environmental impacts of the systems was able to create a broad and nuanced perspective to identify opportunities and trade-offs. This combined approach can therefore help to build a better and more holistic understanding of the agronomic and environmental implications of different management options to enable farmers to make informed decisions, as to both the effectiveness of the practice at managing weeds, pests or diseases, and the environmental implications of applying that tool.

**7.10 To eat, or not to eat? The Guts of PFAS Related Fish Advisories**

**7.10.T-01 Introductory Remarks: PFAS Related Fish Advisories**
***Erin Pulster***[1]*, Christopher J. McCarthy*[2]*, Courtney Carignan*[3] *and Jonathan Petali*[4]*, (1) U.S. Geological Survey, (2) Jacobs Engineering Group Inc., (3) Michigan State University, (4) New Hampshire Department of Environmental Services*
Science-based environmental standards for PFAS vary across regulatory jurisdictions from non-existent to conservative and are constantly changing with emerging science. As each state develops its own consumption advisories, the resulting advisories can seem inconsistent. Measured PFOS levels in fish tissue (0.2 – 200 ppb) spanning over three orders of magnitude and the use of differing toxicity endpoints across states are just some of the factors that can contribute differences in advisories. This session will bring environmental chemists, toxicologists, and epidemiologists together to understand how the evolving science of PFAS impacts fish consumption advisories, as well as to explore cutting-edge research for better informing risk assessment and management. This session will focus on the key areas (epidemiology, analytical challenges, confounding factors, study designs and policy) used in developing PFAS related fish advisories and highlight critical data gaps needed to inform resources managers and risk assessors. The goal for the outcome of this session is to set the foundation for generating recommendations for a path forward towards closing data gaps, achieving consistency, and communicating risk from fish consumption across North America.

**7.10.T-02 Per- and Polyfluoroalkyl Substance and Precursor Bioaccumulation in Freshwater Recreational Fish: Implications for Fish Advisories**
***Heidi Pickard***[1]*, Bridger Ruyle*[1]*, Colin Thackray*[1]*, Adela Chovancova*[1]*, Clifton Dassuncao*[1]*, Jitka Becanova*[2]*, Simon Vojta*[2]*, Rainer Lohmann*[2] *and Elsie Sunderland*[1]*, (1) Harvard University, (2) University of Rhode Island*
Per- and polyfluoroalkyl substances (PFAS) are a diverse class of fluorinated anthropogenic chemicals that include perfluoroalkyl acids (PFAA). Many consumer products and environmental samples contain abundant PFAA precursors that can degrade into terminal PFAA associated with adverse health effects. Several of the PFAA can bioaccumulate in food webs and fish consumption is an important dietary exposure source. However, little is known about bioaccumulation of PFAA precursors. In this work we identified and quantified PFAS in recreational fish species collected from surface waters across New Hampshire, United States using a toolbox of analytical methods. Targeted analysis of paired water and fish muscle tissue samples using liquid chromatography tandem mass spectrometry (LC-MS/MS) suggests that many precursors below detection in water have a higher bioaccumulation potential than their terminal PFAA. Perfluorobutane sulfonamide (FBSA), a short chain precursor produced by the electrochemical fluorination manufacturing process was detected in all

fish samples analyzed for this compound. The total oxidizable precursor assay (TOP) interpreted using Bayesian inference, which groups PFAS precursor classes by their perfluorinated carbon chain-lengths and manufacturing origins, revealed fish muscle tissue contained additional, short-chain precursors in high concentration samples. Suspect screening analysis using a quadrupole time-of-flight tandem mass spectrometer (QTOF MS/MS) revealed these were perfluoroalkyl sulfonamide precursors with three and five perfluorinated carbons. Fish consumption advisories are primarily being developed for perfluorooctane sulfonate (PFOS), but this work reinforces the need for risk evaluations to consider additional bioaccumulative PFAS, including perfluoroalkyl sulfonamide precursors.

**7.10.T-03 Understanding Analytical Interferences in Targeted Per- and Polyfluorinated Alkyl Substances Methods: A Case Study in Shellfish**

***Jacqueline Bangma***[1]*, James McCord*[1]*, Jonathan Petali*[2]*, Kate Buckman*[3]*, Celia Chen*[3]*, Nate Giffard*[3] *and Mark Strynar*[1]*, (1) U.S. Environmental Protection Agency, (2) New Hampshire Department of Environmental Services, (3) Dartmouth College*

The investigation of per- and polyfluorinated alkyl substances (PFAS) in environmental and biological samples relies on both high- and low-resolution mass spectrometry (MS) techniques. While high-resolution MS (HRMS) can be used for identification of novel compounds, low-resolution tandem mass spectrometry (MS/MS) is the more commonly-used and affordable approach for studies examining previously identified PFAS through targeted PFAS analytical methods. To date, targeted studies of PFAS in biological matrices (e.g. blood, serum, egg yolk, placenta, chocolate, etc.) have identified instances where matrix-derived interfering compounds are capable of impacting PFOS, PFHxS, PFPeA, and PFBA quantitation on low-resolution instrumentation. These interfering compounds match the monitored fragmentation patterns of the PFAS under question and can lead to overreporting of PFAS in the literature. Of note, PFPeA and PFBA are two of the smaller PFAS observed in biological and environmental samples, and both have only one major transition for MS/MS monitoring, preventing the use of ion ratios of additional transitions for verification. Therefore, when our lab observed up to 80 ng/g wet weight of PFPeA (263→219 Da) in numerous shellfish samples, we investigated further using HRMS and identified a PFPeA interfering compound at 263.1288 Da and putatively assigned the compound as an unsaturated dicarboxylic acid with a level 3 confidence on the Schymanski scale. With further method development, we were able to establish an additional MS/MS transition for the PFPeA interferent in shellfish samples as well as establish improved chromatographic separation. Therefore, in support of PFAS analysis on low-resolution instrumentation, the authors would recommend additional methods to confirm possible PFAS analytical interferences in biological and environmental samples.

**7.10.T-04 Assessing PFAS in a large urbanized estuary and the potential human health implications**

***Erin Pulster****, U.S. Geological Survey*

The primary source of chronic exposures to per- and polyfluoroalkyl substances (PFASs) in humans is through the ingestion of contaminated foods and drinking water, with fish and other seafood being a major contributor. Nevertheless, there is scant literature on the dietary exposure to PFASs for the general United States (U.S.) population. Tampa Bay supports productive recreational and commercial fisheries, providing a diverse community of species. A number of potential PFAS sources surround Tampa Bay including five airports, light industries known to generate PFASs, numerous wastewater treatment discharge outfalls, several fire-fighting academies and a number of military installations. The objective of this study is to quantify PFASs in sediment and fishes collected from Tampa Bay and estimate human health risks from dietary exposures. Sediment ($n$ = 17) and fish (25 species, $n$ = 140) were collected throughout Tampa Bay and analyzed for 25 PFAS compounds. Concentrations of PFASs in sediments and edible tissues of fish ranged from 36.8 to 2,990 ng $kg^{-1}$ (dry weight, d.w.) and 307 to 33,600 ng $kg^{-1}$ (wet weight, w.w.), respectively. Generally, levels were highest in Old Tampa Bay and decreased south towards the Gulf of Mexico. Profiles in both matrices were generally dominated by perfluorooctane sulfonic acid (PFOS) with variation by location. Estimated human health risks from the consumption of contaminated fish collected in Tampa Bay exceeded concentration thresholds for minimum risk levels (MRLs) and tolerable weekly intake (TWIs) values for adults and youths. Exceedances of thresholds were highest for Old Tampa and Hillsborough Bays and decreased towards the mouth of the Bay into the Gulf of Mexico. Additionally, concentrations of PFOS in edible fish tissues of several recreationally important species collected in Tampa Bay exceeded consumption guideline levels established by several governmental agencies. In the current context, the elevated levels of PFAS in Tampa Bay and the exceedances of available thresholds for potential human health risks are a cause for concern and justify a more intensive examination especially for more heavily utilized species, particularly those used in subsistence-level fishing, which, as elsewhere may be significantly under documented.

**7.10.T-05 Why and How We Should Sample Biota at PFAS-Contaminated Sites in Support of Human and Ecological Risk Assessments**

***Christopher J. Salice***[1] *and Jamie Suski*[2]*, (1) Towson University, (2) EA Engineering, Science, and Technology, Inc., PBC*

Approaches for human and ecological risk assessment of per- and polyfluoroalkyl substances (PFAS) are actively evolving. There are a number of effects-based thresholds and fish consumption advisories. Together, these thresholds are used by risk assessors to estimate risk to ecological receptors during remedial investigations (RI). Importantly, following the 8-step superfund process under Comprehensive Environmental Response Compensation and Liability Act (CERCLA), many PFAS are likely to pass the ecological screening-level thresholds meaning that, functionally, there would be “no risk” to ecological receptors. Hence, there would be no trigger to sample biota such as fish to inform potential dietary exposures to ecological receptors and humans. If we consider perfluorooctane sulfonate (PFOS) in surface waters, measured concentrations often do not exceed ecological effects thresholds; however, PFOS is categorized as highly to very highly bioaccumulative. Thus, despite estimates of fish tissue concentrations at (and below) screening-level effects thresholds, actual concentrations in fish tissues may necessitate fish consumption advisories for humans and a potentially important dietary pathway for ecological receptors.  Using a low surface water screening criteria for PFOS of 0.117 µg/L and a BAF of 1100 L/kg, estimated fish tissue concentrations could be 128 µg PFOS/kg tissue. Fish consumption advisories are generally 20 – 200 µg/kg. Thus, dietary exposure may be erroneously discounted under the current framework of reserving biota sampling for a failed ecological screening threshold and remains an uncertainty for human and ecological receptors. Moreover, recent research at PFAS-contaminated water bodies points to potentially significant variability in PFAS surface water concentrations in space and time. Hence, minimalist sampling designs in which only a few environmental and biota samples are obtained could yield misleading results. As an example, bioaccumulation factors calculated from a small number of samples can range over several orders of magnitude. Until we have a stronger sense of how effects-based thresholds and bioaccumulation of PFAS are related and a better sense of PFAS variability in the environment and its impact on bioaccumulation, we recommend that: sampling for fish be included regularly in assessments of PFAS-contaminated sites and that sampling regimes must consider PFAS variability in environmental media and biota.

**7.10.T-06 Accumulation of PFAS in stocked brook trout in Maine, USA**

***Tom Danielson****, Maine Department of Environmental Protection*

Per- and Polyfluoralkyl Substances (PFAS) are persistent, toxic chemicals that pose a risk to human health.  People can inadvertently consume PFAS when eating fish caught from rivers and lakes.  The Maine Department of Environmental Protection (DEP), Maine Department of Inland Fisheries & Wildlife (IFW), and Maine Center for Disease Control and Prevention started an experiment to determine how quickly stocked brook trout (*Salvelinus fontinalis*) accumulate PFAS in their muscle tissue in the wild.  In October 2021, IFW biologists stocked trout into two small ponds with high concentrations of PFAS in the water.  IFW biologists put 350 fingerlings and 100 yearlings in the larger of the two ponds and 100 fingerlings in the smaller pond.  At the time of stocking, the fingerling trout were almost a year old and the yearling trout were almost 2 years old.  Fish obtained directly from the hatchery had non-detect concentrations of perfluorooctane sulfonate (PFOS) and other forms of PFAS.  The plan was to collect fingerlings from the larger pond 1, 2, 4, 8, 16, and 24 weeks after they were put in the pond. In addition, the plan was to collect yearlings from the large pond and fingerlings from the small pond 4, 8, and 24 weeks after they were put in the ponds. Paired samples of skinless and skin-on fillets were analyzed for PFAS. Concentrations of PFOS in fish tissue were compared to Maine's fish tissue action level (FTAL) of 3.5 ng/g. For most of the study, PFOS concentrations in pond water were >500 ng/L in the large pond and >1,500 ng/L in the small pond. Concentrations of PFOS and other types of PFAS accumulated quickly in the fish tissue and increased over time. PFOS concentrations in fingerlings from the large pond were more than 10 times greater than the FTAL after one week and more than 100 times greater than the FTAL by the end of the study. Fingerlings from the small pond had higher concentrations of PFOS and other PFAS compared to the fingerlings in the large pond. Yearlings from the large pond had less PFOS and other PFAS compared to fingerlings from the large pond. Skin-on fillets typically had more PFOS than skinless fillets. It was not determined how much of the PFAS came from the water or from consumed prey. This study demonstrates that stocked brook trout can accumulate PFOS and other kinds of PFAS quickly when put into waterbodies with high concentrations of PFAS in the water.

### 7.10.T-07 Seafood as a source of per- and polyfluoroalkyl substance exposure among residents of New Hampshire

***Megan Elizabeth Romano***[1]*, Nate Giffard*[2]*, Lisa Gallagher*[1]*, Kathryn Crawford*[3]*, Tracy Keirns*[4]*, Sujan Fernando*[5]*, Jonathan Petali*[6]*, Thomas Holsen*[5] *and Celia Chen*[2]*, (1) Geisel School of Medicine at Dartmouth, (2) Dartmouth College, (3) Middlebury College, (4) University of New Hampshire Survey Center, (5) Clarkson University, (6) New Hampshire Department of Environmental Services*

Per- and polyfluoroalkyl substances (PFAS) are persistent, fluorinated chemicals used in a wide variety of industrial and commercial applications with known adverse health effects. PFAS pose a regional as well as global threat to surface water and drinking water. PFAS in seafood is believed to be an underappreciated dietary source of exposure. The northeastern United States (US) is home to many PFAS-contaminated sites and seafood consumption rates are higher than other regions of the US. We conducted a population-based survey of New Hampshire (NH) residents (n=1,829) to determine frequency of seafood consumption and typical portion size among adults and children aged 2-11 years old residing within surveyed households. Among adult respondents, 93% reported seafood consumption within the last year, 94% in the prior month, and 77% had within the previous week. Overall, shrimp, haddock, salmon, and canned tuna were the most frequently consumed types of seafood. Adult respondents most frequently reported that fish portions consumed were ~5 ounces, whereas the average portion size for children was ~ 2 ounces. Approximately a quarter of the respondents (28%) resided in a household in which at least one member had a NH fishing license within the prior five calendar years. Almost half (44%) of respondents indicated that some of the seafood they consumed in the past year was caught by someone in their household from NH waters; common species caught in NH waters included black crappie, bluegill, pickerel, and walleye fish. The majority of respondents (68%) indicated that their fish consumption habits were not influenced by the COVID-19 pandemic. PFAS concentrations in fish tissues were generally low, but several PFAS were detected in >70% of the fish sampled [(perfluorooctanesulfonic acid (PFOS), perfluorotetradecanoic acid, perfluoroundecanoic acid (PFuDA), perfluorotridecanoic acid, perfluorohexadecanoic acid, perfluorodecanoic acid, perfluorododecanoic acid, perfluorononanoic acid]. Across species sampled the highest average concentrations of PFAS were observed for PFuDA (mean=0.27 ng/g; standard deviation=0.19) and PFOS (mean=0.24 ng/g; standard deviation=0.37). Estimated daily intake of PFAS from fish were calculated using the combined survey and lab data and the findings will inform future risk assessment activities.

### 7.10.T-08 Best practices for human health risk assessment associated with PFOS in fish

***Evelyn G. ReateguiZirena****, Gregory J. Garvey, Philip E. Goodrum and Janet K. Anderson, GSI Environmental, Inc.*

Fish consumption advisories are used by state agencies within the United States to limit consumption of contaminated fish for the protection of human health. Advisories not only include recommended limits on the number of fish meals and types of species consumed over time, but also the preparation and cooking methods. For example, many fish consumption advisories for polychlorinated biphenyls (PCBs) recommend removing the skin and trimming fatty portions of the fish as an additional means to limit exposure by removing the more contaminated portions of the fish prior to cooking. This study evaluated the methodologies and inputs used by state agencies for recommended fish advisories for perfluorooctane sulfonate (PFOS) in freshwater fish. To date, states have focused on PFOS ahead of other PFAS because of its frequency of occurrence in watersheds, relatively higher bioaccumulation potential in fish, and relative potency. We conducted a side-by-side comparison of PFOS fish consumption advisories issued by state agencies across the U.S. and highlight key differences in the exposure and toxicity values used to support the final advisory levels. While differences in selected toxicity values for PFOS are relatively straight forward to evaluate, minor differences in conventions used to define consumption rates can also have significant impacts on the minimum concentration in fish tissue that would trigger an advisory. These conventions apply to PFOS as well as other commonly targeted contaminants (e.g., PCBs, methyl mercury, lead). We also examined the sensitivity of advisory levels if a cooking loss value for PFOS were applied. We summarized the available scientific literature that reports changes in PFOS mass or concentration as a function of not only the size, fat content, and species of fish, but also the fillet preparation and cooking methods. Given that preparation and cooking methods are already a component of some advisories, the additional protection afforded by accounting for cooking loss for PFOS provides opportunities to revisit best practices for setting advisory levels and/or communicating the degree of health protectiveness of advisory levels.

**7.10.P To Eat, or Not to Eat? The Guts of PFAS-Related Fish Advisories**

**7.10.P-We196 Bioaccumulation potentials and Ecological impacts of Per- and polyfluoroalkyl substances (PFAS) exposure to Recreational fisheries in offshore Biscayne Bay, Miami, Florida.**

***Olutobi Daniel Ogunbiyi Sr.****, Richard Brinn and Natalia Soares Quinete, Florida International University*

Per- and polyfluoroalkyl substances (PFAS) are regarded as a group of synthetic, manmade chemicals prominent for their ubiquitous, persistent, and bioaccumulative properties in all environmental compartments. Legacy PFAS especially PFOA and PFOS have been commonly reported in aquatic organisms due to their strong proteinophilic properties. This makes their ecotoxicological impacts on the aquatic habitat of great concern. According to the NOAA fisheries, Florida ranks first in the number of recreational anglers' fishing trips. Thus, Florida is termed "the fishing capital of the world". Recreational fisheries such as blackfin tuna (*Thunnus atlanticus*) and lobsters (*Homarus americanus*) are greatly sought by recreational fishermen in South Florida. Hence, exposure to environmental emerging contaminants such as PFAS can affect the ecological health of aquatic organisms in this region and could represent a human health risk through seafood consumption. Biscayne Bay is a unique marine estuary located in Miami-Dade County, and naturally endowered with seagrass and coral reef ecosystems, with a lot of fishing activities; especially offshore of the southern barrier islands are hot spots for recreational fishers. Blackfin tuna (N=15), and lobsters (N=18) were caught using hook and line while surface water samples (N=20) were collected using 500 mL polypropylene bottles. The muscles were processed using an alkaline based extraction followed by a dispersive solid phase extraction as clean-up (dSPE) and instrumental analysis using LC-MS/MS. The total PFAS concentration was 14.43 ng/g, 21.32 ng/g and 112.26 ng/L for Tunafish, lobsters, and water samples, respectively. The bioaccumulation (BAF) of long chain PFAS ($\geq 8$) was higher in blackfin tuna than in lobsters while PFTeDA was the highest in lobsters. However, C7, C12 and C11 PFAS congeners were not bioaccumulated in lobsters. Principal component analysis (PCA) was applied to further understand the variability of PFAS data obtained, indicating distinct patterns likely associated with different feeding habits and trophic levels. Furthermore, morphometric indices were assessed to determine the Fulton's condition factor (FCF) of 2.5-3.8, suggesting that PFAS might not be affecting the fisheries' health and physiological conditions. Human health risks due to consumption of Tunafish and lobster were also evaluated to establish safe quantities for fish ingestion.

**7.10.P-We197 PFAS in Colorado Fish: Policy Development and Community Engagement**

***Kelsey Barton***[1]*, Kristy Richardson*[1]*, Sarah Choyke*[2] *and Christopher Higgins*[3]*, (1) Colorado Department of Public Health and Environment, (2) Eurofins Environment Testing (EET), (3) Colorado School of Mines*

In 2020, the Colorado Department of Public Health and Environment partnered with the Colorado School of Mines and Colorado Parks and Wildlife for a pilot project aimed at evaluating per and polyfluoroalkyl substance (PFAS) concentrations in fish. We collected 49 fish samples across 10 species from three Colorado water bodies and analyzed them for a suite of 48 PFAS. The intent of this pilot project was to inform future sampling direction and to evaluate the need for PFAS fish consumption advisory development to reduce PFAS exposure for Colorado residents. Results demonstrated that recreationally-caught fish could represent an important source of PFAS, and in particular perfluorooctane sulfonate (PFOS) exposure, in Colorado. Here we will discuss these preliminary findings and evaluate the implications they have for future policy development in Colorado. Additionally, we review how this project highlighted both challenges and opportunities associated with community awareness and risk communication, and changes to our community engagement approach moving forward. Though this project was small and had some limitations, the information gained has proven valuable as we consider how to move forward in protecting Coloradans from PFAS exposure via all pathways.

**7.10.P-We198 Per- and polyfluorinated alkyl substances (PFAS) in finfish and shellfish from the Great Bay Estuary and the Gulf of Maine**

***Nate Giffard***[1]*, Jonathan Petali*[2]*, Megan Elizabeth Romano*[3] *and Celia Chen*[1]*, (1) Dartmouth College, (2) New Hampshire Department of Environmental Services, (3) Geisel School of Medicine at Dartmouth*

Per- and polyfluorinated alkyl substances (PFAS) in finfish and shellfish, particularly in freshwater ecosystems, are recognized as an important dietary source of exposure. However, data regarding exposures from marine sourced seafood are limited, hindering the development of public health guidance. In the northeastern United States (US), where an abundance of PFAS contamination sites have been identified near aquatic ecosystems and seafood consumption rates tend to be high relative to other regions of the US, marine species comprise a considerable share of the market. Little is known about PFAS concentrations in the region's finfish and shellfish, and preliminary data show some novel PFAS compound profiles in bivalves not detected in earlier studies conducted in the region. Moreover, the spatial extent of tissue concentrations in finfish and shellfish near contaminated sites have not yet been evaluated nor have concentrations in coastal seafood from nearshore vs. offshore areas. We investigated a suite of twenty-seven common and novel PFAS compounds in 1) demersal finfish species from the nearshore and offshore areas of the Gulf of Maine; and 2) commonly consumed marine shellfish species (i.e., clams, mussels, and oysters) near the former Pease Air Force Base. The project utilized an archive of existing finfish samples from the Gulf of Maine and recently collected oyster, mussel, and clam samples from Great Bay (GB) in areas near and far from the PFAS contamination site where AFFF has been found in surface water and groundwater. Filet tissue from each individual finfish were homogenized and whole, shucked shellfish samples from 10 individuals were pooled and homogenized. Tissues were extracted using methods developed by Clarkson University and analyzed using LC-MS/MS. Data on the PFAS concentrations and compound profiles will be presented and discussed for finfish and bivalve species in relation to their distances from PFAS sources and their food sources and feeding strategies. In addition to the value that these data have for monitoring PFAS remediation efforts around Great Bay, our study will aid state and local governments in keeping finfish and shellfish advisories up to date with current PFAS science. Updates to these PFAS related advisories will ensure the health and safety of New Hampshire residents living around Great Bay, as well as provide the State of New Hampshire with more targeted goals for local remediation efforts.

## 7.11.P Late Breaking Science: Policy, Management and Communication

**7.11.P-Tu194 What You Can Do With AIST-MeRAM: An All-In-One Tool for Multi-Purpose Ecological Risk Assessment and Management (MeRAM) of Chemical Substances**

***Bin-Le Lin****, National Institute of Advanced Industrial Science and Technology, Japan*

A quality-assured ecological risk assessment (ERA) requires enormous resources (time and labor) in collection/assessment of hazard data, as well as considerable expertise to interpret the risk. The ERA of chemicals is thereby considered difficult or impossible for those with little assessment experience and cumbersome or complicated for practitioners. To meet the concerns regarding ERA and accelerate the risk assessment and management of chemicals, we developed an all-in-one free tool for multi-purpose ecological risk assessment management (MeRAM) of chemical substances in aquatic environment called the AIST-MeRAM Ver. 2.0.0 (Copyright No: H28PRO-2007). It allows users from beginners to experts to conduct ERA without any preparation because all the necessary ecotoxicity test data and methodologies are available in the system. Approximately 270,000 ecotoxicity test data points for 3900 chemical substances together with the scientific methodologies from traditional simple hazard quotient (HQ) to more ecologically relevant complicated assessments such as species sensitivity distribution (SSD) and population-level assessment are embedded in the AIST-MeRAM. In addition, users can easily understand the Japanese regulatory RA and management of chemical substances due to a special function based on the Japanese Chemical Substance Control Law (CSCL). Here, we demonstrate a tiered ERA using the embedded sample data to evaluate and ensure the functions of AIST-MERAM. We show that the AIST-MeRAM can provide a comprehensive and accurate ERA, suggesting that it is a powerful IT solution for cumbersome ERA.

**7.11.P-Tu195 Simple, Confusing or Sensible – Differences in Communication of Uncertainty in a Conclusion of a Scientific Assessment**

***Ullrika Sahlin***[1] *and Andy Hart*[2]*, (1) Lund University, Sweden, (2 )A&A Hart Ltd*

The answer to an assessment question will always be uncertain to some degree, and this uncertainty is what matters most to decision makers. We compare strategies to characterise and communicate uncertainty in conclusions developed by three organisations conducting scientific assessments: the Intergovernmental Panel of Climate Change, the Cochrane Collaboration with applications in evidence-based medicine, and the European Food Safety Authority. Their strategies differ with respect to how uncertainty in a conclusion is expressed: quantitatively, qualitatively or a mixture of both. There are commonalities in the principles and methods to identify and consider the combined impact of all relevant sources of uncertainty when characterising uncertainty in a conclusion, where differences can be due to the nature of their assessments.

Uncertainty can as a concept be challenging to understand and fully embrace as part of a scientific process by experts engaging in scientific assessments and decision makers relying on scientific advice.

It is important to develop strategies to communicate uncertainty in conclusions based on conceptual clarity and evidence from communication research. Acknowledge the subjective nature of uncertainty, someone is uncertain and the best we can do is to characterise it as honestly as possible. Adopt scientific approaches to use expert judgements to consider all sources of uncertainty, i.e. not only those that are addressed by the assessment model or a statistical analysis. Engage decision makers in the development of strategies to communicate uncertainty in conclusions. Find ways of expressing uncertainty are understandable and useful to them, however without compromising the need to communicate scientific uncertainty in conclusions. Evidence shows that qualitative expressions of uncertainty, such as likely or unlikely, are ambiguous and mean different things to different people. For example, this is why EFSA's approach is to recommend that assessors always try to quantify the overall uncertainty quantitatively using probability. This should always be possible, provided the conclusion is well defined, but can be challenging for those unfamiliar to the approach. Clarify the link between specific expressions for uncertainty in a conclusion and the scientific assessment by documenting the methods, evidence and reasoning for the assessment is essential for transparency and credibility of the conclusion.

**7.11.P-Tu196 California and Beyond: A Survey of State and Federal Approaches to Microplastic Regulations**

***Shannon Edmonds****, Rachel Henke, Rachel Maxwell and Jon Rohrer, Roux*

California is paving the way for microplastics regulation and analytical method development in the United States. In September, California finalized accredited drinking water analytical methods and a sampling handbook to guide a five-year drinking water sampling program. By legislative directive, California is 1) implementing a standard testing method, 2) developing a five-year program requiring annual testing in drinking water, 3) considering the need for quantitative health-based guidelines, and 4) developing a statewide strategy to mitigate risk to ecological receptors and coastal ecosystems by 2025. Nationally and globally, governmental agencies, policy makers, stakeholders, and NGOs have expanded initiatives to understand the potential risks, identify knowledge gaps and develop strategies for best management practices of microplastics.

Following California's implementation of a standard analytical and testing methodology, it is expected that Illinois and several other states will follow suit in implementing regulations to further characterize and/or regulate microplastics in the environment. To forecast future action, our analysis presents various upstream and downstream initiatives and regulations that are currently proposed or have been adopted (ranging from downstream investigation requirements to upstream single use plastic bans).

Lastly, using water purveyors in California as an example, we will explore how these regulations are expected to be practically applied and discuss potential consequences in the regulated community.

### 7.11.V Late Breaking Science: Policy, Management and Communication

### 7.11.V-01 Derivation of Federal Water Quality Guidelines for Benzene, Toluene, Ethylbenzene, and Xylenes

***Kathleen McTavish*** *and Sushil Dixit, Environment and Climate Change Canada*

Federal Environmental Quality Guidelines (FEQGs) are benchmarks for the quality of the ambient environment that support various Canadian federal activities including risk assessment, risk management, and environmental quality monitoring. Environment and Climate Change Canada is currently deriving long-term guidelines and short-term benchmarks for benzene, toluene, ethylbenzene, and xylenes (BTEX) for freshwater and marine surface waters. These long-term guidelines represent concentration thresholds below which there is low likelihood of adverse impacts from BTEX on aquatic species. Monitoring data can be compared against the guideline values to inform site screening, risk assessment and/or risk management. This poster will showcase the various information gathered and approaches used to derive the draft guidelines including the target lipid model (TLM) and species sensitivity distributions (SSDs). Uncertainties and next steps will also be presented.

## Track 8: Systems Approaches

### 8.01 Advances in Methods, Policies, and Practices for Safer and More Sustainable Alternatives

### 8.01.T-01 Regulatory Alternatives Assessments that Lead to Successful Chemical Substitution

***Catherine A Rudisill***[1]*, Molly Jacobs LeFevre*[2]*, Holly Davies*[3]*, Timothy Malloy*[4]*, Monika A. Roy*[2] *and Lauren Brown*[5]*, (1) Safer Chemistry Advisory LLC, (2) University of Massachusetts, Lowell, (3) Washington State Department of Health, (4) University of California, Los Angeles, (5) ToxStrategies, Inc.*

Regulatory restrictions on chemicals of concern have been increasing across local, state/provincial, federal, and international levels. Some restrictions are promulgated from overarching chemical management frameworks, like REACH, which evaluates alternatives in the context of authorizations. Others are modeled directly around the use of chemical alternatives assessment (AA) (e.g., California Safer Consumer Products). As alternatives assessment is further incorporated into regulatory practice, it is critical that policy is crafted in a way that achieves the ultimate goal of avoiding regrettable substitutes. In April 2021 the Association for the Advancement of Alternatives Assessment (A4), a professional association dedicated to advancing the science, practice, and policy of AA and informed substitution, convened a discussion group consisting of experts within its community of practice representing government, consulting, and academia. That discussion group sought to better understand how these regulatory policies conform to AA best practices. The discussions focused on lessons learned from recent regulatory actions and development, considerations for integrating AA into regulatory programs, and key challenges to successful implementation. These discussions also included two sessions at the A4 International Symposium on Alternatives Assessment held in October 2021. The group identified specific challenges to successful chemical substitution covering three main areas: the scope of statutory authority and responsibilities, agency and industry capacity, and data disclosure and transparency. The challenges are illustrated in the context of 4 case studies covering REACH authorization, the California Safer Consumer Products program, Washington State's Safer Products for Washington, and EPA's Significant New Alternatives Policy program. The discussion group has developed recommendations for future policy development covering the incorporation of AA into legislation and future development of AA methodology and implementation.

**8.01.T-02 Building effective alternatives assessment infrastructure - Discussion**
***Catherine A. Rudisill***[1]*, Molly Jacobs LeFevre*[2] *and Monika A. Roy*[3]*, (1) Safer Chemistry Advisory LLC, (2) University of Massachusetts, Lowell, (3) University of Massachusetts, Amherst*
This discussion session will engage the audience by asking: What is needed to push chemical alternatives assessment beyond a tight-knit community of practitioners to building technical standards, methodologies, and pedagogy to firmly establish it as a sustainability practice?

**8.01.T-03 Determining the Feasibility and Availability of Alternatives in State-led Alternatives Assessments: Case Studies from Washington State**
***Rae Eaton***[1]*, Marissa Smith*[1]*, Saskia VanBergen*[1]*, Craig Manahan*[1]*, Sascha Stump*[1] *and Holly Davies*[2]*, (1) Washington State Department of Ecology, (2) Washington State Department of Health*
Background: Alternatives assessment is a process used to identify, compare, and select alternatives to chemicals of concern based on their human and environmental health hazards, ability to meet technical performance requirements, and economic viability. In Washington State, we use these assessments to support regulatory decisions about chemicals of concern used in everyday consumer products. Challenge: Unlike many alternatives assessments, the goal of our assessments is not to identify the most preferable alternative but instead to demonstrate that preferred alternatives exist within the market. These assessments are conducted by technical experts within the state government and largely rely on publicly available or voluntarily submitted information, which informs what strategies are used. This presentation focuses on our evaluation the technical performance, or feasibility, and availability of alternatives. Results: We developed an approach to evaluating technical performance and economic viability that reflects the goals and realities of our assessments. This presentation will discuss the challenges of evaluating the feasibility and availability of alternatives and present case studies that demonstrate our approach.

**8.01.T-04 Filling Data Gaps for Chemical Hazard Assessment in Alternatives Assessments: Analogue Selection Using a Multifactor Approach**
***Mary Kawa****, Courtney Hard, Lauren Cassidy, Savannah Sierco, Laura Morlacci and Christina Coley, SRC Inc.*
Comparative hazard assessment is a key component of chemical alternatives assessments. However, many chemicals are missing experimental data for important endpoints to support chemical hazard assessments. Selection of appropriate analogue chemicals to fill these data gaps helps identify potential hazards and improves confidence in selection of safer chemical alternatives. Analogue selection is critical to the success of this approach. Suitable analogues must be justifiable on the basis of expected similarity in their chemical hazards relative to the target chemical. The analogue selection tool methodology, along with the physical-chemical properties of the analogue(s) and the chemical being assessed need to be considered when identifying analogues. Suitable analogues typically have similar physical-chemical properties as the chemical being assessed since these properties often inform behavior *in vivo* and in the environment. Chemical analogues can

be collected based on structural similarity, structural fragments, functional class and/or potential for a shared mechanism of action (MoA) with the target chemical. Each analogue identification approach has benefits and drawbacks and each tool has different underlying datasets. Using only one tool may omit critical candidate analogues; therefore, to address this issue a multipronged approach to analogue selection using multiple tools increases the pool of analogues for evaluation and better ensures that as many analogues as possible are considered. This assessment will describe the use of the currently understood best practices, and free, publicly available tools that support analogue selection for alternatives assessment: OECD QSAR Toolbox, EPA CompTox Chemicals Dashboard, Hazard Comparison Dashboard, ToxRead, ToxAlerts, ChemIDplus, NTP Integrated Chemical Environment, OPERA and EPA's ChemACE, AIM, and EPI Suite™ and will demonstrate the collection and selection of suitable analogues for molecules of interest by evaluating chemical structures, physical chemical properties and MoA.

**8.01.T-05 The ChemFORWARD SAFER Program and Safer Trade Name Ingredients**

***Chris Bartlett**, Lauren Heine and Stacy Glass, ChemFORWARD*

Alternatives assessment involves comparing the hazard profiles of chemicals with similar functions to choose the chemical with the least inherent hazard. Chem*FORWARD* is a not for profit organization (NGO) that houses a centralized online platform of chemical hazard assessments (CHAs) designed to support users in selecting safer chemical ingredient alternatives. The purpose of Chem*FORWARD* is to provide comprehensive, credible, and actionable hazard profile information so that users can compare chemicals with similar functions and select those that are well characterized for hazard and inherently safer than existing chemicals, which is critical to avoiding regrettable substitutions. Botanicals and polymers present unique challenges in hazard characterization due to their lack of traditional toxicology data, making them difficult to assess in a manner similar to traditional chemicals. To overcome this challenge we have developed an assessment approach to evaluate and score botanicals and polymers in the ChemFORWARD repository, in a manner that allows comparison with traditional chemicals. The ChemFORWARD platform was initially designed to identify safer chemicals by CAS number and other generic modifiers but we have since developed the SAFER program to evaluate ingredients by trade name. Trade name ingredient assessments consider additives, residuals and impurities in the hazard review process, aspects that can vary between manufacturers. While this is important for all chemicals, it is particularly important for botanicals and polymers, which can vary based on manufacturing processes. The ideal process for selecting a safer alternative would be to determine a potentially safer chemical alternative and then find a supplier that produces it without additives or impurities of concern. This presentation will provide an overview of the ChemFORWARD CHA repository and SAFER program and provide the necessary background for a case study assessing polymeric materials as safer alternatives.

**8.01.T-06 A Trade Name Market Differentiator: Case Studies under the ChemFORWARD SAFER Program**

***Jiaru Zhang**[1], Charlotte Marsh[1], Kim Reynolds Reid[1] and Patrick Harmon[2], (1) Gradient, (2) BASF Corporation*

Although many existing alternatives assessment schemes revolve around discrete chemicals, in reality, the selection of alternatives often involves an assessment of trade name ingredients and products that may contain intentionally and/or unintentionally added substances, such as stabilizers and impurities. While these substances often exist at relatively low concentrations, their presence may significantly affect the hazard profile of an ingredient or product, and therefore should be accounted for when evaluating potential human health and environmental impacts. The Chem*FORWARD* SAFER program was developed to allow manufacturers and formulators to identify safer ingredients by *trade name*, rather than CAS number, based on a third-party review of all substances within an ingredient at or above 100 ppm as well as any brand-specific certifications or regulatory approvals. This presentation will provide an overview of the SAFER hazard assessment process, including how the program considers impurities, monomers, catalysts, residuals, *etc*. We will present case studies for plasticizer and polymer ingredients that received the SAFER designation, discuss the underlying data used to develop the profiles, and demonstrate how the process builds on approaches of other frameworks such as the GreenScreen® for Safer Chemicals and the US EPA Safer Chemicals Ingredients List (SCIL). Ultimately, the SAFER qualification for trade name ingredients and products will be integrated into platforms such as Covalo and ChemSEC, allowing companies to compare ingredients/products and make well-informed material selection and substitution decisions.

**8.01.T-07 Eliminating 6PPD from tire manufacturing: Computational efforts toward safer antiozonants**
***Elliot Rossomme**, University of California, Berkeley*
Elimination of 6PPD (*N*-[1,3-dimethylbutyl]-*N'*-phenyl-*p*-phenylenediamine) from tire manufacturing became a pressing problem in chemical alternatives with the discovery that 6PPD quinone, an ozonation product of 6PPD, possesses acute toxicity to coho salmon and other aquatic species. Despite widespread interest, identification of safer alternatives has proven formidable due to the combination of physical and chemical properties that allow 6PPD to protect rubber compounds from degradation due to oxygen radicals and ozone while preserving the mechanical properties of tires. To date, identification of safer candidates to achieve the antioxidant chemistry of 6PPD has been relatively successful, while our understanding of the ozonation chemistry of rubber compounds and the mechanism of 6PPD antiozonant activity remains relatively limited. This is troubling, as the toxicity of 6PPD is directly linked to its function as an antiozonant. We report the use of tools in computational quantum chemistry to better understand the mechanism of 6PPD's antiozonant function in rubber compounds in an effort to identify alternative antidegradants and evaluate their reactivity towards ozone. With functional insights from these mechanistic studies, we develop a methodology for high throughput screening of the technical and environmental feasibility of alternative antiozonants. In the course of this work, we emphasize the role of collaboration across academic, government, and industrial organizations in pursuing safer alternatives to existing chemistries.

**8.01.T-08 Understanding Lessons Learned from Efforts to Accelerate the Substitution of Safer Alternatives to Aqueous Film Forming Foam**
***Molly Jacobs LeFevre**[1], Melissa Lavoie[2], Joel Tickner[1], Pam Eliason[3] and Elizabeth Harriman[3], (1) University of Massachusetts, Lowell, (2) Northeast Waste Management Officials' Association, (3) Toxics Use Reduction Institute*
Aqueous film forming foam (AFFF) is a highly efficient fire suppressant agent used for Class B fires. The FY 2020 National Defense Authorization Act requires the U.S. Department of Defense (US DoD) to phase out use of Aqueous Film Forming Foam (AFFF) products contains per- and polyfluorinated alkyl substances (PFAS) at military installations by October 1, 2024. The purpose of this research project was to explore lessons learned from efforts to accelerate the adoption of substitutes for PFAS-free alternatives to AFFF as adoption experience is an under-addressed topic in alternatives assessment practice. Using literature reviews and key informant interviews with stakeholders in the U.S., Europe and Australia, the research team sought to enhance understanding of: (1) critical technology, market and policy factors (actual and perceived) that are inhibiting the implementation of alternatives to AFFF; (2) critical technology, market and policy factors that are enabling and scaling a transition to safer and feasible alternatives to AFFF; and (3) lessons learned from US DoD's current efforts to accelerate the adoption of safer and effective AFFF alternatives that can improve efforts to address future material challenges. This presentation will review findings associated with these three research objectives. Examples of barriers to be discussed in this presentation include transition costs, standards, and the lack of a coherent national substitution strategy. Examples of enabling factors to be discussed include policy, use of "real-world" performance tests and the use of fit-for-purpose performance standards. Notable lessons learned to improve adoption of substitutes for future chemical/material challenges to be discussed include the importance of training and education, the use of collaborative performance testing programs and the need to establish systems for ongoing monitoring and evaluation.

**8.01.P Advances in Methods, Policies, and Practices for Safer and More Sustainable Alternatives**

**8.01.P-Th150 Combined Application of the Essential-Use and Functional Substitution Concepts: Accelerating Safer Alternatives**

***Monika A. Roy***[1]*, Ian Cousins*[2]*, Elizabeth Harriman*[3]*, Martin Scheringer*[4]*, Joel Tickner*[1] *and Zhanyun Wang*[5]*, (1) University of Massachusetts, Lowell, (2) Stockholm University, Sweden, (3) Toxics Use Reduction Institute, (4) ETH Zürich, Switzerland, (5) Empa – Swiss Federal Laboratories for Material Science and Technology*

Faced with the near-impossible task of assessing and regulating chemicals of concern on an individual-basis, governments need rapid and effective decision-making approaches for chemicals management. Adapted from the Montreal Protocol, the Essential-Use Concept (EUC) was proposed in 2019 as a policy approach to expedite the phaseout of "non-essential uses" of chemicals of concern not necessary for the "health, safety, or critical functioning of society". The EUC is now a key element of the European Commission's Chemicals Strategy for Sustainability (CSS), where it is currently undergoing further clarification and implementation into chemicals legislation. However, the CSS does not define "alternatives from the standpoint of environment or health" in a clear and explicit manner. Here, we contribute to ongoing conversations around the implementation of the EUC, specifically regarding the incorporation of the Functional Substitution concept, which was built into the EUC in 2021. This viewpoint further explores the intersection of the two concepts and notes that to effectively support the transition to safer and more sustainable chemicals, materials, products, and processes – a key goal of the CSS – the EUC needs to be accompanied by Functional Substitution. Functional Substitution was first described in 2015 and calls for the identification of the functional use of a chemical of concern, evaluates if the function is necessary for the application, and then examines whether safer and effective chemical, product/process design, or product service alternatives exist to fulfil that function. The contribution of Functional Substitution to the EUC is that it expands the range of alternative options considered, evaluated, and compared *to meet the specific technical function needed for an application*. **Used together, the Essential-Use and Functional Substitution concepts form a complementary and more solutions-oriented approach to chemicals management**. This presentation provides an overview of the two concepts and outlines at a high level where Functional Substitution thinking could be useful in Essential-Use decision-making, providing some action steps that might result from the merging of the two concepts. In addition to supporting the implementation of the CSS in EU policy, a combined approach may create a "substitution and innovation mindset" that could also be applied in regulatory and non-regulatory settings for identifying safer solutions in a timely manner.

**8.01.V Advances in Methods, Policies, and Practices for Safer and More Sustainable Alternatives**

**8.01.V-01 Identifying Safer Alternatives to Lead-Contaminated Aluminum Cookware**

***Stephen George Whittaker***[1]*, Katie M. Fellows*[1]*, Yoni Rodriguez*[1,2] *and Shar Samy*[2]*, (1) Hazardous Waste Management Program in King County, (2) University of Washington*

An investigation into the cause of lead poisoning in Afghan refugee children resettled in King County, Washington revealed that aluminum cookpots and pressure cookers from Afghanistan are a previously unrecognized source of lead exposure. We subsequently learned that aluminum cookware the Afghan community might purchase in the United States is also contaminated with lead. To understand the contribution of this cookware to childhood lead exposures, we developed a novel leachate method to determine how much lead would be released under simulated cooking and food storage conditions. We also tested stainless steel cookware, to determine whether stainless-steel is a safer alternative. We found that many imported aluminum cookpots and pressure cookers leached enough lead to exceed the Food & Drug Administration's (FDA's) Interim Reference Level (IRL) for children, including all cookware donated by Afghan families. In contrast, no stainless-steel cookware leached enough lead to exceed the IRL. We conclude that stainless-steel is a safer alternative to some imported aluminum cookware. To address immediate consumer concerns, we recommend selection/sourcing of cookware certified by NSF International (Standard P390: Stovetop Cookware for Home Use), which specifies maximum leachate levels for several toxic substances, including lead. These findings have informed several interventions, including providing safer stainless-steel cookware to Afghan families in King County and outreach campaigns to other vulnerable communities and stakeholders.

**8.01.V-02 Assessing Safer Alternatives to Priority Chemical Classes**

***Holly Davies***[1]*, Marissa Smith*[2]*, Craig Manahan*[2]*, Saskia VanBergen*[2]*, Sascha Stump*[2]*, Rae Eaton*[2]*, Lauren Tamboer*[2]*, Elinor Fanning*[1] *and Cheryl Niemi*[2]*, (1) Washington State Department of Health, (2) Washington State Department of Ecology*

This presentation will describe our criteria for identifying safer alternatives to classes of hazardous chemicals in products and the challenges we've faced to implement our new state law. In 2019, the Washington State Legislature passed the Pollution Prevention for Healthy People and Puget Sound Act. The Department of Ecology's Safer Products for Washington Program implements this law in consultation with the Department of Health. Instead of conducting single chemical and product risk assessments, Safer Products for Washington focuses on reducing the use of classes of hazardous chemicals by identifying safer alternatives. Evaluating chemicals by class avoids the problem of treating chemicals with insufficient data as not hazardous and can prevent regrettable substitutions. This law defines safer alternatives as being less hazardous to people or the environment than the existing chemical or process. Since the existing chemical or process is a class of chemicals, we aimed to determine whether alternatives are less hazardous than the priority chemical class. The challenges we faced included how to define less hazardous, how to assess a class of chemicals instead of individual chemicals, and how to deal with hazard differences within a class. We developed specific hazard criteria to define a hazard spectrum with specific data requirements. The criteria are based on EPA's Safer Choice and GreenScreen® for Safer Chemicals criteria, which both rely on the Global Harmonization System. We rely on the data rich chemicals within a class for the hazard assessment. When there is variation within the data rich chemicals, there are several options for assessment in our decision framework. This approach has allowed us to identify safer alternatives to priority chemical classes.

**8.02.P Material Flows in the Circular Economy: Environmental Impacts and Sustainability Implications**

**8.02.P-Tu180 Environmental and Human Health Impacts of Enhanced Rock Weathering for Carbon Capture**

***Danyi Feng*** *and Andrea Hicks, University of Wisconsin, Madison*

The concentration of global carbon dioxide (CO2) in the atmosphere has increased 12% since 2000. Therefore, immediate actions are required to meet the target established in the 2015 Paris Agreement that limits global temperature increases between 1.5 °C to 2 °C. Several negative emission technologies (NETs) that have been mentioned by the Intergovernmental Panel for Climate Change (IPCC) to prevent increasing CO2 levels in order to reach climate stabilization in the future. Enhanced weathering (EW) is one of the NETs that has attracted an increasing amount of attention in recent years. The principal of EW is to utilize pulverized silicate mineral rocks (e.g., wollastonite, basalt) to sequester CO2 from the atmosphere, and then transport and permanently store the final dissolved products (e.g., CaCO3) in the deep ocean layer. EW not only impacts the global carbon cycle due to the elevated uptake of atmospheric CO2, but also has high potential for nutrient runoff reduction, productivity improvement and soil water retention enhancement in agriculture land. However, the capacity of EW for carbon capture depends on the application area, type of rock selected, silicate composition, particle size of rock, application rate, and comminution technique. A comprehensive literature review presents current studies about life cycle assessment (LCA) of EW for carbon capture. The purpose of this study is to recognize and address critical research gaps through the understanding of various environmental and human health impacts associated with a variety of silicate rocks, application rate of silicate minerals, transportation, and geographic application area. Key findings are (i) the main contributor to environmental impacts is transportation for all impact categories; (ii) the highest application rate of powdered rock can capture more CO2; (iii) the climate change impact for remaining processes except electricity and transportation are negative which means the sequestered CO2 is higher than the concentration of CO2 emitted through EW.

**8.02.P-Tu181 Healthcare Sustainability, Disposable vs Reusable Speculums for Pelvic Examinations: A Case Study at UW Madison Using a Life Cycle Assessment Approach**

***Monica Rodriguez** and Andrea Hicks, University of Wisconsin, Madison*
Pelvic examinations are a common part of women's healthcare. Medical providers use vaginal speculums to conduct these examinations. Speculums are available in reusable and disposable materials, and where this study was conducted, the providers decided which kind of speculum to use. The environmental impacts of both types of speculum systems, reusable stainless-steel speculums and disposable acrylic speculums, were determined through life cycle assessment (LCA), using a functional unit of one year of operation in the UW Madison women's health clinic, which is equivalent to 5,000 pelvic exams. The reusable speculum system includes the water and detergents used for soaking, washing and rinsing; the electricity used to run the autoclave; the personal protective equipment (PPE) used by personnel to handle the speculums; the materials used to make the stainless-steel speculums; and associated wastes from the disinfection and final speculum disposal. The disposable acrylic speculum system consisted of the plastic film used to package the speculum and the acrylic speculum itself. There are no use phase impacts for the disposable speculum system. In the use phase analysis of the reusable stainless-steel speculums, most of the environmental impacts arise from nitrile glove production, polyethylene gown production, and electricity originating from coal. The reusable speculum had less environmental impacts in five impact categories: acidification, fossil fuel depletion, global warming, respiratory effects, and smog. If considering uncertainty, there are 4 impact categories where there is no system with better outcomes: carcinogenics, ecotoxicity, eutrophication and non-carcinogenics. In the ozone depletion impact category, the reusable system performs worse. The higher impact arises from the production of the nitrile gloves used during speculum disinfection for reuse. The type of system recommended depends on the clinics' environmental goals, and other aspects such as access. In the case of the UW Madison clinic, reusable speculums are now mostly used to reduce the clinic's global warming impacts.

**8.02.P-Tu182 Nano-specific impact factors for usage in life cycle assessment: nano-silver as a case study**
***Andrea Hicks** and Sila Temizel Sekeryan, University of Wisconsin, Madison*
Engineered nano-materials have the potential to transform everyday life through their added functionality when compared to the bulk counterparts. At the same time, they have to potential to differ in environmental impact from their bulk counterparts. Often, the life cycle assessments of these materials use surrogates, such as ionic forms of the bulk counterpart. This is due to the complexity of and effort needed to produce the nano-specific characterization and impact factors for the materials. Nano-scale silver (nAg) is utilized in this work to illustrate the potential to 1) calculate nano-specific characterization and impact factors, 2) compare these factors to their ionic surrogates, and 3) apply these factors to life cycle assessment. The toxicity of the nAg is dependent on not only the shape, size, and coating of the particle itself, but also the receiving environment. This work seeks to explore wether calculating nano-specific impact factors is necessary in life cycle assessment for nAg.

**8.02.P-Tu183 Novel Approach to Normalization in Life Cycle Assessment Based on Natural Constraints**
*Jan Matuštík, **Aleš Paulu** and Vladimír Kočí, University of Chemistry and Technology Prague, Czech Republic*
There are currently multiple human-driven environmental crises. In order to successfully navigate and manage these crises, we need tools to evaluate human impact on the environment, such as the Life Cycle Assessment (LCA) method. The multiple environmental issues are in LCA represented by multiple impact categories expressed in different units. The step of normalization is employed to allow comparison of results of these impact categories. This is currently done by referencing the impacts of the evaluated system to the cumulative impact on the global or regional level. However, this approach does not consider the limits of the Planetary system, and thus it does not reflect the overall sustainability. We propose an alternative approach to normalization based on environmental references. The new normalization factors are based on quantifiable natural constraints, rather than the unsustainable cumulative human impacts. We developed these factors for each of the impact categories included in the currently most policy-relevant LCA methodology Environmental Footprint 3.0. The rationale for designing these limits was inspired by the concept of Planetary boundaries. Nevertheless, instead of a permissible harm in the safe operating space for humanity, we chose a no-harm approach based on natural absorption capacities. To give an example, the environmental reference for Climate

change impact category is based on the Earth's total greenhouse gas sink. We hypothesize that such normalization factors would influence the interpretation of LCA results and potentially lead to different conclusions. The relevance and functionality of the new approach, in comparison to the current one was tested and verified on a case study. The strength of this way to combine LCA with planetary limits lies in the fact it can be directly applied to an established and familiar LCA methodology. We believe this novel approach to normalization allows more relevant evaluation of sustainability of the analyzed systems and thus would better support environmental management.

**8.02.P-Tu184 Human Behavior When Disposing of Polylactic Acid Cups: Waste Audits For Informing Life Cycle Assessment End of Life Scenarios**

***Monica Rodriguez**, Audrey Stanton, Travis Blomberg and Andrea Hicks, University of Wisconsin, Madison*

Bioplastics have been suggested as an opportunity to reduce the environmental impacts of plastic usage. Inventories of some lifecycle stages of bioplastics are well-quantified in the literature, such as raw materials and manufacturing, while the end-of-life stage is primarily incorporated as stylized scenarios. The life cycle assessments (LCA) in the literature make assumptions based on regional availability of waste management infrastructure, but centering people, who use and dispose products, is needed to create scenarios that reflect real world behaviors and conditions. Overall, these scenarios should consider available infrastructure, properties of the material, and user disposal behavior. User disposal behavior was addressed in this research. Waste audits were conducted at the UW–Madison campus during the 2021-2022 academic year. Five waste audits were completed, each on a different day. The waste sorted for the first three waste audits came from the same university building. This building is multipurpose and houses dining facilities, a movie theater, a recreation center, and meeting rooms. The waste for the fourth and fifth audits was from residence halls. **Waste audit findings reveal that people probably do not know or do not act on what they know when disposing of PLA cups.** In addition, people were also inconsistent in their sorting of other types of plastic cups such as PETE, PP and PS. However, in the case of PLA, disposal behavior is most erratic. Data from waste audits can be incorporated into the end-of-life stage in an LCA and may lead to better modeling and society-level forecasts as to the comparative environmental impacts of bioplastic usage.

**8.02.P-Tu185 Material Flow Analysis of Per- and Polyfluoroalkyl Substances within the Automobile Recycling Stream in the U.S.**

***Erin Bulson** and Andrea Hicks, University of Wisconsin, Madison*

Automobiles are comprised of a variety of materials, many of which include per- and polyfluoroalkyl substances (PFAS). However, the environmental fate of PFAS derived from end-of-life vehicles is not fully understood. PFAS are synthetic, organic chemicals that have been widely used since the 1940s for their many beneficial properties, such as high heat and corrosion resistance. PFAS also play an important role in lightweighting vehicles to achieve reduce emissions and fight climate change. However, PFAS are also highly mobile in the environment and bioaccumulate, and some have been linked to harmful impacts to human health such as cancers and early childhood neurodevelopmental disruption. Recent material flow analyses for PFAS in waste streams have included end-of-life vehicles, with a focus on the European Economic Area. Automobile shredder residue (ASR), the non-recyclable fraction remaining after vehicles are shredded for recycling, has been projected to contain increased amounts of PFAS into the future. In addition, several studies have identified potential environmental pathways and impacts for PFAS related to auto recycling and derived from ASR, as indicated by industrial runoff and groundwater data. Therefore, it is critical to evaluate ASR-derived PFAS from both a sustainability and environmental impacts perspective. Through our materials flow analysis, our research aims to improve understanding of the stocks and flows of PFAS within the vehicle recycling stream and associated waste within the United States. Findings from our material flow analysis may aid in development of policy and best management practices for ASR as it relates to PFAS.

**8.02.P-Tu186 Ecotoxicological characterization of sludge originated from olive oil wastewater treatment: potential to be use in agriculture**

*Andreia Pereira, Amid Mostafaie, José Nereu Pinto, Rita Silva, Diogo Filipe Nunes Cardoso, Amadeu Soares and **Susana Loureiro**, University of Aveiro, Portugal*

The valorization and re-use of waste and circular solutions are crucial for a more sustainable world, contributing to mitigating climate change scenarios. Based on that, the project NETA - "New Strategies for WasteWater Treatment" (Portugal 2020) was created to valorize different wastewaters, by transforming them into valuable resources. In the last year, the world's olive oil production tripled and, consequently, overproducing of olive oil industry wastewaters has been increasing, highlighting the demand for environmentally friendly solutions for its storage and disposal. Aware of that, the chemical precipitation technique (CPT) has been recognized as a possible solution to treat wastewaters, generating expected less hazardous wastewaters and sludges. Further, this sludge can be applied directly onto agricultural land or pass through a biological treatment using insect larvae. This larvae ingest sludge and produce frass that can be thus peletized into a biofertilizer (entofertilizer). Considering this, this study aimed to test if sludges originated from olive oil wastewater treated with CPT can be applied to soils with no deleterious effects and if a further passage through insect bioreactors should be carried out. For that, ecotoxicological tools were employed, using different sludge application rates in Lufa 2.2 soil, evaluating plant performance (*Brassica oleracea* and *Lolium perenne*) and the effects on soil invertebrates (*Folsomia candida and Enchytraeus crypticus*, adult survival and reproduction). Considering the rates usually applied for these sludges into soils, fewer effects were observed for plants compared to soils with no sludge application. Adult survival of soil invertebrates was not affected by the presence of sludge in the soil at the tested concentrations; nonetheless, the number of juveniles produced decreased significantly at 2% (mimicking recommended/realistic field dosages), 4%, and 8% of sludge in soils. Therefore, further evaluation and treatment should be considered for this type of sludge, with ecotoxicology assessment demonstrating being a complementary tool for hazard assessment of sludge for soil application.

**8.02.P-Tu187 Example Framework for Chemical Additive Replacement Prioritization in a Circular Economy for Plastics: Human Health Perspective**

***Jennifer Bare**, Stephanie Vivanco and Julie Panko, ToxStrategies, Inc.*

Plastics have many beneficial uses throughout consumer and industrial sectors, and often have complex product designs for the intended use. However, managing plastic waste has several challenges that has led to a high prevalence of plastic pollution in the environment and landfilling of plastics instead of recycling or reuse. Global production of plastics is expected to reach 1.2 billion tonnes by 2050; therefore, moving toward a circular economy for plastics has become a global initiative to combat plastic pollution and waste problems. The European Commission (EC) has developed a circular economy strategy for plastics that highlights several research and innovation priorities. Here, we focus on the substances of concern to human health and the use of safer and more circular alternatives. We present a prioritization framework that was developed to assist in evaluating chemical additives in a plastic product. The framework is based on functional use, circularity potential, and human exposure potential categories. Each category has several parameters that are scored on a scale of 1 to 3, where the higher score indicates a higher priority to replace. The parameter scores are weighted by a confidence rating of 1 to 3, corresponding to high to low confidence (i.e., higher confidence lowers the prioritization score). The weighted sum can be used to prioritize chemicals to replace, as well as an indicator for selecting alternatives. A simplified prioritization score for an example additive is calculated as a demonstration. This framework may be dynamic, to add more parameters or adjust scores as research continues, and it may even be adapted for chemical groupings as outlined by the EC as a research priority. Integration of this framework with an existing regulatory hazard prioritization framework would be necessary to inform health risk potentials. Use of the framework is subjective at the discretion of the evaluator and works best with data-rich chemicals, or those for which physical-chemical parameters can be modeled. Further, while the framework is currently tailored toward human health, the ecological health perspective will be considered in future versions, although a different scoring approach may be needed. While the circular economy strategy for plastics is complex, this approach will be helpful in addressing the issues surrounding substances of concern.

## 8.03.P Methods, Measures, and Models for Characterizing Restoration Effectiveness

### 8.03.P-Mo176 Hydro-Geological Conceptual Site Model Refinement Using a Groundwater-Surface Water Interface (GSI) Trident Probe.

***Joel M. Guerrero***[1]*, Bradley Davidson*[1]*, James Leather*[1]*, Nicholas Shih*[2]*, Ed Long*[2] *and Carly Spencer*[2]*, (1) Naval Information Warfare Center (NIWC) Pacific, (2) Naval Facilities Engineering Command Southwest Division*

Historical investigations at a naval installation restoration (IR) site adjacent to an underground storage tank (UST) showed several monitoring wells with elevated petroleum hydrocarbon constituents (TPHs) in groundwater near the San Diego Bay shoreline. Site generated contour data from the wells identified potential groundwater plume discharge areas in the bay next to the site. The possibility of exposure to ecological receptors at the groundwater-surface water interface (GSI) is a concern.

A porewater investigation was conducted to identify potential TPH venting zones and determine if TPH concentrations in sediment pore water potentially pose ecological risks to aquatic biota. The survey involved the collection of porewater for TPH analysis using the Trident Probe-- a direct-push, integrated conductivity/temperature (C/T) sensor and GSI sampler. The investigation involved pore water sampling at multiple inshore and offshore locations in a grid pattern and directly opposite the areas of elevated onshore groundwater TPHs. Laboratory pore water analytics, complemented by low (brackish) water quality conductivity measurements confirmed the presence of detectable TPH concentration over the regulatory screening level in only one single location.

Overall, the Trident Probe data provided critical information needed to refine the conceptual site model (CSM) regarding the relationship of site groundwater discharge potential with the GSI. Subsequently, pore water data and results were used for a coastal contaminant migration monitoring (CCMM) application to evaluate potential ecological risks, support ongoing inshore site characterization and remediation activities, guide a focused monitoring assessment along the shoreline, and to augment and optimize follow-up corrective actions.

### 8.03.P-Mo177 Ecological Effects of Iron in Non-restored Streams and Regenerative Stream-water Conveyance Systems

***Megan Gaesser****, Sarah Lanasa and Christopher J. Salice, Towson University*

Regenerative Stream-water Conveyance (RSC) systems are a restoration method utilized to address poor water quality draining urban catchments. It has been hypothesized that the construction of RSC systems may increase dissolved iron concentrations, precipitate, and flocculate in Anne Arundel County (AAC), Maryland streams via several pathways. It is important to note that iron is naturally occurring in AAC streams and sediments given the underlying geology. Increased iron may impact benthic biota of these streams by coating and imbedding into aquatic habitat as well as increasing indirect and direct toxicity, but this is poorly understood. Ecosystem functions, such as nutrient processing and decomposition, may also be affected by this change in structure (e.g., altered macroinvertebrate and microbial activity). This study aims to elucidate the biological and ecological impacts of iron in eight RSC systems and seven reference streams through water quality monitoring, and toxicological and ecological field experiments. Monthly water quality monitoring was used to characterize the conditions of RSC systems versus reference streams. In situ macroinvertebrate enclosures were deployed and leaf packs will be deployed to evaluate the toxicological response of caddisfly larvae and to assess the decomposition of organic matter, respectively, under various iron conditions. We hypothesize that iron concentrations and toxicity will be higher in RSC systems while leaf pack decomposition will likely be lower. Preliminary results of the environmental monitoring indicate that most of the study stream's average iron

concentrations exceed the chronic criterion for iron set by the EPA and that iron concentrations are higher in RSC systems. Additionally, survival of hydropsychid caddisflies in caged field experiments show an apparent decrease in survival associated with higher iron concentrations. Potentially exacerbated iron conditions as an unintended consequence of RSC restoration may have important implications for future restoration programs in AAC, in particular. This research applies field experimentation and survey methods to address concerns regarding RSC implementation and subsequent iron conditions.

**8.03.P-Mo178 Characterizing Grassland Management Practices for Ecological Health Investigations Using Remote Sensing Methods**

***Jillian LaRoe***[1], *Christopher M Holmes*[1] *and Thorsten Schad*[2], *(1) Applied Analysis Solutions LLC, (2) Bayer CropScience LLC*

Grasslands support essential biodiversity and ecosystem services. Their conditions are strongly shaped by the type and intensity of anthropogenic use. Remotely sensed satellite imagery has previously been utilized to characterize the intensity and usage of grasslands over time and broad geographic areas. We used synthetic aperture radar (Sentinel-1) and optical multispectral imagery (Sentinel-2) to identify grassland types and management practices at a high temporal resolution. Intensely managed grasslands may be cut multiple times per season. Timeseries images were used to characterize grassland type, cutting frequency, and use intensity for over 6,000 grassland parcels in Germany between 2018 and 2021. Spectral indices related to vegetation were derived across grassland patches to create annual metrics. Thresholding methods were applied to annual time series of satellite imagery to detect cutting events within each grassland patch. Results from the two methods were combined to improve cut detection confidence, and a random forest model was used to classify the grassland type and use intensity. Comparable approaches have been used to develop landscape scenarios for honeybee risk assessment and health monitoring, select and characterize habitat for wood mouse (*Apodemus sylvaticus*) population models for regulatory pesticide risk assessment, and to characterize insect habitat. The final statistics provide a suite of metrics that may be useful for assessing the quality of insect habitat, pollinator forage distribution in space and time, and investigating which grassland management practices best support biodiversity. Furthermore, these methods could be refined for monitoring grassland use and intensity of use over time for designing integrated agricultural management strategies (e.g., integrated weed control strategies in context to landscape ecosystem services' conditions) or assess restoration effectiveness.

**8.04 New Tools and Wise Perspectives: Advancing Environmental Assessment and Management Through Reflection**

**8.04.T-01 What I Learned the Hard Way about Assessment Innovations**

***Glenn W. Suter II***

After 45 years as an environmental assessor for both a responsible party (Department of Energy) and a regulatory agency (Environmental Protection Agency--EPA), I know things that I wish I had known, and I will share some of them. 1. Fancy innovative methods are seldom adopted. When a group at Oak Ridge National Laboratory was asked by the EPA to develop an equivalent of human health risk assessment, we created probabilistic models for organisms, populations, and ecosystems. They were technically impressive, and the publications helped launch our careers, but they were not used in regulatory practice. No manager wants to go up to court with “there is a 63% probability that the effluent caused impairment.” Further, even clearly advantageous innovations are resisted if they are perceived as complex and difficult. 2. Species sensitivity distributions are a model innovation. 3. Conceptual advances are more important than technical. 4. Human health dominates, even when ecological effects are clearer and more sensitive. Keep assessing ecological risks but relate them to ecosystem services. 5. Weight of evidence is an essential tool. Use it explicitly. 6. Watch for needs expressed by regulators or practitioners. For example, how can I use in vitro data in assessments? 7. Make yourself useful.

**8.04.T-02 Assessing chemical risks under the European Water Framework Directive, a prosaic reality.**
***Graham Merrington****, Dean Leverett and Adam Peters, wca environment limited, United Kingdom*
The European Water Framework Directive is a unifying piece of legislation that has brought together aspects of water resources, water management and water quality. In considering water quality, chemicals are identified that present a Europe-wide risk, across more than 25 countries. For these chemicals a legally binding Environmental Quality Standard (EQS) is set, that must be complied with across all countries. These EU-wide risks are initially identified and assessed using relatively crude PEC (or MEC) to PNEC ratios.

Clearly, more advanced, and sophisticated approaches exist to make these assessments; tools do currently exist to account for chemical speciation, bioavailability, and uptake. Frameworks of assessment are available to ensure both effects and chemical exposure data are relevant and reliable. Yet, from 20 years of experiences with the chemicals risk assessment under the WFD, it is apparent that if we are to deliver improvements in risk assessment in regulatory contexts then efforts focussed upon pragmatic, practical delivery are critical to produce step changes in our approaches. However, such efforts are extremely unlikely to gain research grants, win science prizes or get the most reads in high impact journals, meaning they remain largely orphaned issues. The gap between novel technicques in risk assessment and environmental benefit is unbridgeable without such efforts. If we cannot make the existing, accepted approaches deliver consistency and transparency, what hope is there for those novel approaches?

Such an example under the WFD is the consideration of bioavailablity for metals, which has been legally in place for nearly 10 years. Yet, at least a third of European countries do not measure the supporting parameters to implement the EQS correctly. Indeed, the staggering long gestation period (> 25 years) between scientific delivery of biotic ligand models and routine regulatory consideration of bioavailability.

Step changes in our chemicals risk assessment approaches also requires revisiting the aim of the driving purpose. To deliver environmental benefit there is a need to understand change in highly complex, highly variable, systems and be able to communicate this change by means of readily understandable and repeatable metrics. The framing and contextualisation of risks and clear recognition of the implications of decisions, linked to these metrics of environmental benefit are therefore required.

**8.04.T-03 Data Accessibility: The Next Frontier for Environmental Science and SETAC**
***Ryan R. Otter****, Middle Tennessee State University*
Data accessibility is not a singular idea, it refers to a variety of activities currently being implemented across multiple science disciplines, including those represented at SETAC. For example: the unlocking of achieved records using data science tools (e.g., extraction of data from PDFs), the use of advanced software techniques that aide with data exploration and complex data sets (e.g., interactive visualizations), and the inclusion of data management plans and raw data repositories for funded studies and peer-reviewed publications. The implications of implementing these data accessibility solutions are significant and will lead to new discoveries and non-siloed scientific inquiry, but they will also present new challenges that need to be addressed (e.g, embracing new technology and/or team members with differing expertise). In this presentation, current practical solutions and challenges surrounding data accessibility will be addressed, as well as the implications of these changes on current and future scientists.

**8.04.T-04 Discussion - New Tools and Wise Perspectives: Advancing Environmental Assessment and Management Through Reflection**
***Adam C. Ryan**[1], Frannie Nilsen[2], Ralph G. Stahl Jr.[3] and Tom Augspurger[4], (1) International Zinc Association, (2) NC DEQ, (3) DuPont, (4) U.S. Fish and Wildlife Service*

**8.04.T-05 Environmental Risk Assessment in the Tropics - Disasters, Innovations and Quality Control**
***Ross Smith**, Gisele Kruger and Dustin Hobbs, Hydrobiology*
Over the last 25 years or so, we have been involved in responses to environmental incidents in several tropical countries. A commonality of those responses to complex issues has been a lack of baseline data, which has always required carefully constructed and robust sampling designs to identify the impacts to and recovery trajectories for the receiving ecosystems against the background of other pressures and stressors. The fundamental principle of hypothesis-testing designs, and stringent quality control of datasets is old-school science, but is no less critical now than it was decades ago.

In many tropical countries, which are known to support high biodiversity, often considered to be a critical national asset, but generally have limited baseline data on species distributions and sensitivity, it is generally assumed that the potential for impacts from environmental incidents is high. Lack of government investment across STEM sectors also commonly results in a strong emphasis on biodiversity and taxonomic assessments of ecosystems, not environmental risk assessment, and regulatory and practice inertia that effects in-country assessment capability.

Nonetheless, innovations in data collection strategies, new ecosystem health assessment tools, and weight of evidence frameworks for impact assessment have greatly helped us with improving inference for the risk assessment. Perhaps counter-intuitively, a lack of regulatory specificity has often allowed us to use leading-edge approaches and tools such as remote sensing, acoustic surveys, environmental genomics, site specific guideline value development and targeted ecotoxicity testing, provided those approaches were well supported by the literature. We have found that the Australian and New Zealand implementation of the iterative water and sediment quality framework and use of weight of evidence provides the flexibility needed for sound risk assessment for highly complex issues with little baseline information in other regions too. The specific inclusion of indigenous cultural and spiritual values is also beneficial.

**8.04.T-06 A Retrospective Look at Regulatory Whole Effluent Toxicity Data: Can we learn more from the data?**
***Michelle Rau Embry**[1], Sarah A. Hughes[2], Carolyn Martinko[3], Ryan R. Otter[4], Camille Flinders[5], Leana Van der Vliet[3] and Teresa J. Norberg-King, (1) Health and Environmental Sciences Institute (HESI), (2) Connecticut Agricultural Experiment Station, (3) Environment and Climate Change Canada, (4) Middle Tennessee State University, (5) NCASI, Inc.*
Before Rachel Carson published her book Silent Spring, few governments had regulations to protect water bodies and aquatic life from toxic effects from chemical discharge. Increased scientific awareness and public concern about water pollution in the 1970s led to dramatic developments in the number and type of aquatic toxicity test methods and in turn, many jurisdictions worldwide have developed regulations for effluents, and some countries have incorporated requirements into their laws. Environmental regulations on effluent quality have controlled the release of key sector-specific contaminants. Intense efforts into the standardization of test methods first led to whole organism acute toxicity test methods (e.g. 48 h, 96 h tests with a variety of species) and short-term tests (96 h to 8 days) were developed to predict chronic toxicity for both freshwater and marine waters. These standardized aquatic toxicity test methods for effluents include fish, invertebrates and algae that share the characteristics of reliability, repeatability, and reproducibility. Fast forward many years and the advances in manufacturing and wastewater treatment technologies that were driven largely by regulatory agencies and stakeholder demands that discharges do not adversely affect water quality, biota, or human health have subsequently improved the quality of municipal and industrial effluents. Improved effluent quality and public pressure to reduce the number of vertebrates used in toxicity assessments has encouraged a re-evaluation of existing toxicity evaluation programs (e.g., number of species, number of replicates in dose response testing) and the development of New Approach Methodologies (NAMs) that include the concepts of refining, reducing, replacing biological methods for acute and chronic endpoints while still achieving water quality objectives. In

this presentation, we will discuss opportunities to draw from decades of knowledge gained from regulatory aquatic toxicity testing to evaluate and refine existing toxicity evaluation frameworks and reimagine existing approaches. Specifically, we present case studies where trend analysis of historical regulatory data led to regulatory changes beneficial to the industry, improved environmental monitoring, and/or provided insights into the path toward regulatory acceptance for NAMs. We will also discuss how data collected during culturing and testing of standardized toxicity test species can be used for method improvement, new species test method development, and improved interpretation of sub-lethal organism responses. Finally, we will describe the challenges of harnessing existing data for evaluating trends and propose potential paths forward to improved data collection and data sharing.

**8.04.T-07 Information Flow Analysis: A Method for Connecting Relevant Study Areas to Each Other and to Decision Making**

***Valentina Helen Pauna*** *and Cecilia Askham, Norwegian Institute for Sustainability Research NORSUS, Norway*

Interdisciplinary collaboration in the context of environmental assessment remains challenging. Despite the persistent research interest in marine microplastic (MP) particles, the pollutant is still largely misunderstood. Significant data gaps hinder experts' understanding of the sources, pathways, and fate of marine MPs, making it difficult to assess its environmental implications. Interdisciplinary work is required to fully understand the complexity of marine MPs (MMP) and to address the potential stress that this form of pollution may put on marine ecosystems. We introduce an approach called Information Flow Analysis (IFA) which intends to connect different fields of study through their data flows, demonstrating the importance of these fields in the development of Life Cycle Impact Assessment (LCIA) of MMPs. This was based on a specific case study where the IFA approach was used to clarify expected microplastic data sources with the goal of providing insight on how to obtain the data required for Risk Assessment (RA) and Life Cycle Assessment (LCA). A coarse Material Flow Analysis (MFA) demonstrated that MP sources in that specific fjord area of Norway were in line with the data provided from blue mussels sampled by the Norwegian Institute for Water Research (NIVA). This complemented work on data availability and data quality for micro- and nanoplastic sampling in an interdisciplinary team of scientists working with environmental sampling and monitoring, RA and LCA (including LCIA).

Jargon and lack of adequate understanding of the needs for harmonisation and metadata causes barriers between experts from diverse academic and professional backgrounds and specializations, hindering progress that the scientific community needs to address today's global environmental problems. We propose that these barriers can be overcome and our IFA shows that reliance on data and information provided by diverse fields of study to demonstrate relevant, critical, and symbiotic connections among fields can be part of the solution. Information flow analysis can connect disciplines in a holistic approach, to facilitate the communication of the value of the interconnectivity between fields and how data can be shared and exploited to achieve a greater common utility of these valuable resources.

**8.04.T-08 Bioinformatics: A new frontier for extrapolating toxicity knowledge to protect the diversity of species in the environment**

***Carlie LaLone****, U.S. Environmental Protection Agency*

The application of powerful, pharmacology-based computational methods to inform ecotoxicological chemical safety decisions would have seemed relatively far-fetched 60 years ago. However, with advances in sequencing technologies, sharing of big data on publicly accessible platforms, and the development of multi-disciplinary systems approaches to address complex environmental challenges, our abilities to make use of existing and expanding knowledge of biological pathways has significantly expanded. Consequently, the use of bioinformatics is a rather new concept to make its way into supporting chemical safety relative to the protection of environmental health. Tools that facilitate gene/protein sequence comparisons across species for understanding chemical susceptibility are expanding and the use of such data for decision-making, particularly in a world where animal testing is becoming more limited, is steadily entering mainstream discussions of regulatory toxicology. Web-based applications like the US Environmental Protection Agency's Sequence Alignment to Predict Across Species Susceptibility tool is one such approach that has received attention for its utility in predicting chemical susceptibility across hundreds to thousands of untested species rapidly, providing evidence of structural conservation that can be applied to define the taxonomic domain of applicability of an adverse outcome pathway. Further, there have been demonstrated use cases in ecotoxicology of applying more advanced computational approaches such as molecular modeling, molecular docking, molecular dynamics simulation, and virtual screening that show promise to expand the understanding of chemical-protein interactions to inform chemical safety. Although unimaginable when Rachel Carson wrote Silent Spring, the advancement of these computational approaches and their demonstrated uses in cross species extrapolation are charting a new path in environmental assessment that has the potential to lead to more efficient chemical screening and a reduction in the use of animals in toxicology. *This abstract neither constitutes nor necessarily reflects USEPA policy.*

**8.05.P Software and Database Development and Application: Toward Interoperability for Knowledge Synthesis**

**8.05.P-We200 Development of a Curated, Cross-Species Androgen Receptor Database**
***Sara M. Vliet****, Scott G. Lynn, Kristan Markey and Carlie LaLone, U.S. Environmental Protection Agency*
The US Environmental Protection Agency (US EPA) is tasked with assessing chemicals for their potential to adversely impact human and environmental health. This often involves generating toxicity data for model organisms and extrapolating effects to species of concern. For ecological assessments, extrapolation to thousands of diverse organisms is necessary since testing within each species is simply not feasible. Further, limited resources and global efforts to reduce animal use make it challenging to meet demands for chemical testing. One potential solution is to utilize data already present in toxicological literature. This project develops a curated, cross-species database on the androgen receptor (AR) using an artificial intelligence-assisted systematic literature review to categorize *in vitro* and *in vivo* AR data of ecotoxicological relevance. To identify applicable literature, searches were conducted in both PubMed and Web of Science using query expansion technologies. Using web-based software to create evaluation templates, reviewers screened 3,216 *in vitro* and 1775 *in vivo* articles. Articles were initially screened by title and abstract for relevance followed by a full-text screening, which ensured certain criteria for data extraction were met and studies with critical deficiencies were excluded. Articles with relevant, high-quality data underwent data extraction for basic study design and effect information. At each screening stage, discrepancy analyses were conducted to identify disagreements between model and human reviewer responses. This process was assisted by machine learning models trained to identify potential disagreements. Following completion of the systematic review, 33 *in vitro* and 245 *in vivo* articles underwent complete data extraction, including articles containing data for 54 chemicals designated as AR pathway reference chemicals. Across *in vitro* articles, most extracted toxicity data were for fish species (25) with fewer articles for other non-mammalian taxa (5 avian, 2 amphibians, and 1 reptile). Similarly, the *in vivo* articles contained more fish data (196 articles) than for other taxa (20 avian, 29 amphibians, and 1 reptile). This database provides a scientific resource for a myriad of purposes including the validation of new testing methods and assessing the concordance of mammalian-based AR results with other species and assay platforms. *The views expressed in this abstract do not necessarily reflect the policies of the US EPA.*

**8.05.P-We199 ToxCast's invitroDB: Software and database enhancements to support continued integration and use of in vitro screening data**
***Madison Feshuk****, Sarah E. Davidson-Fritz, Katie Paul Friedman, Jason Brown and Richard Judson, U.S. Environmental Protection Agency*
Bioactivity data can be utilized in a tiered screening strategy for next generation risk assessment. The US

Environmental Protection Agency Toxicity Forecaster (ToxCast) program makes *in vitro* medium- and high-throughput screening assay data publicly available for the prioritization and hazard characterization of thousands of chemicals of interest. The assays included employ a variety of technologies to evaluate the effects of chemical exposure on diverse biological targets from distinct proteins to more complex cellular processes like mitochondrial toxicity, nuclear receptor signalling, immune responses, and developmental toxicity. The ToxCast data pipeline (tcpl) is an open-source R package that stores, manages, curve-fits, and visualizes ToxCast data as well as populating the linked MySQL Database, InvitroDB. In the InvitroDB v3.5 release (Summer 2022), the number of publicly available data has increased with new endpoints related to steroidogenesis, cardiotoxicity, and acute and developmental neurotoxicity, with updates in assay annotations to enable better data aggregation. The InvitroDB v4.0 release will include the same data as v3.5, but reprocessed with tcpl v3.0 in an updated database schema to accommodate the addition of more curve-fitting models. Seven additional models (linear and quadratic polynomial, power, and four exponential variants) based on BMDExpress2 and encoded by the R package dependency, tcplFit2, have been added to the previous constant, Hill, and gain-loss models available in tcpl v2. Along with bidirectional curve-fitting, tcpl v3.0 will provide tcplFit2 model parameters, continuous hit call probabilities, and interactive, yet consistent visualization of concentration-response curves within a new utility called tcplPlot. Tcpl and InvitroDB provide a standard for consistent and reproducible data management and curve-fitting for diverse *in vitro* assay data with readily available documentation, thus enabling interoperability and use. These enhancements will support the continued utility of ToxCast *in vitro* screening results as a resource for bioactivity data in myriad toxicology applications. *This abstract does not necessarily reflect U.S. EPA policy.*

## 8.05.V Software and Database Development and Application: Toward Interoperability for Knowledge Synthesis

### 8.05.V-01 Application of Interspecies Correlation Estimation (ICE) Models in EPA's Risk Assessment Frameworks

***Sandy Raimondo****, Karen Eisenreich, Mike Elias, Kellie A. Fay, Mark Jankowski, Kara Koehrn, Andrea LaTier and Crystal Lilavois, U.S. Environmental Protection Agency*

A significant challenge in exposure effects assessments conducted for chemical evaluations and deriving protective ecological hazard values is the limited amount of data available for evaluating a chemical’s toxicity to the diversity of taxa found in the environment. Species sensitivity data are often limited to standard surrogate species, but may be absent entirely for some chemicals. Often, chemical evaluations either cannot be completed or are based solely on the measured or modeled (e.g., quantitative structure activity relationships) sensitivity of just a few species. In these cases, hazard thresholds are typically calculated using a single most sensitive value, an approach that is heavily reliant on the amount of data available. Interspecies Correlation Estimation (ICE) models are log-linear regressions of the acute toxicity tested in two species. ICE models are developed from a standardized database of diverse species sensitivity and represent the relationship of inherent sensitivity of the two species. In application, the sensitivity of a surrogate species (represented by the x-axis) is entered into the ICE model to estimate a value of the predicted species, genus, or family (represented by the y-axis). There are 4000 ICE models available on the US EPA’s Web-ICE application (www3.epa.gov/webice) that predict to aquatic vertebrates and invertebrates, algae, terrestrial mammals and birds. From this application, a few surrogate species can estimate toxicity to over a hundred diverse taxa. As the international ecological risk assessment community of practice moves away from whole animal testing and towards New Approach Methodologies, ICE models have great potential to increase toxicity data available for a diversity of taxa that can inform chemical evaluations. This taxonomic expansion allows for probabilistic approaches of calculating hazard thresholds and data-driven ways to consider uncertainty. In this presentation, we demonstrate the application of ICE models within the US Environmental Protection Agency’s risk assessment framework and highlight examples supporting the Toxic Substances Control Act, the Clean Water Act, and the Endangered Species Act. The views expressed in this abstract are solely those of the authors and do not represent the policies of EPA. Mention of trade names or commercial products should not be interpreted as an endorsement by EPA.

## 8.05A Software and Database Development and Application: Toward Interoperability for Knowledge Synthesis

### 8.05A.T-01 Standardization and Streamlining of Quality Control (QC), Data Provenance, and Data Source Cataloging Workflows Using the Data Accuracy Tool (DAT)

***Jonathan T. Wall****, Carl Frederick Valone, Nelson D'Silva, Joshua Powell, Manli Chan and Amar Singh, U.S. Environmental Protection Agency*

Common challenges for data curation efforts are the ability to quickly review the accuracy of extracted records and to easily catalog data sources to maintain data provenance. This is imperative to ensure reusable authoritative datasets from curated data sources. The challenges further compound when datasets are collaboratively curated and shared across organizations, each having gone through a different workflow, using different quality tags or fields, and using different data source management systems. The Data Accuracy Tool (DAT) is a browser-based quality control (QC) and data source cataloging application designed to enhance the capability of checking the accuracy of curated data irrespective of data type, data domain, or storage schema. DAT was an output of the TSCA QC Tiger Team that highlighted the various challenges to assessing the data quality of their assigned chemical substance datasets and accessing primary sources for data provenance. They identified an urgent need for specialized curation/quality control resources, common data dictionaries, standardized quality flags, and a data provenance system. The purpose of the DAT is to meet these needs by providing a standardized means of QC and data provenance, which will improve the trust and reliability of reviewed data and enhance the ability to share and compare datasets across organizations. The DAT provides a single collaborative interface for managing and tracking the progress of a dataset's QC efforts through a standardized workflow. Dataset managers upload dataset records in batches, create and manage their pool of reviewers, allocate records for review with deadlines, and generate reports and visualizations. Reviewers work through a queue of dataset records based on their expertise, perform side-by-side record source to extracted data reviews, flag records with standardized QC flags, take QC notes by record, edit records with a full audit trail, and associate data sources to records. Due to the underlying relational database and containerization of datasets into key-value record subsets, data from any discipline or project may be easily integrated and reviewed using the DAT application. Although the DAT is currently an internal EPA Office of Research and Development application, it is aspired to expand to broader partnerships within EPA and beyond. *This abstract does not necessarily reflect EPA policy.*

### 8.05A.T-02 EPA's CompTox Chemicals Dashboard: Making Connections for You

***Nisha Sipes****, U.S. Environmental Protection Agency*

Start with the EPA's CompTox Chemicals Dashboard to discover toxicological data and resources for over 900,000 chemicals. The Dashboard connects a wealth of chemistry, in vivo (human health and ecotoxicology relevant), in vitro, and exposure and functional-use databases for browsing and to download, links to several maintained and curated resources (including the AOP-Wiki, SeqAPASS, ECOTOX, EPA's Toxics Release Inventory), and contains applications to run real-time chemical property and read-across predictions and literature searches. This application is publicly available and free to use, with various navigation routes depending on the stakeholder's use-case. For example, stakeholders searching a well-studied chemical may find data in most areas of the Dashboard, including exposure and safety information, presence in known chemical lists, and human and ecological information. Stakeholders evaluating data-poor chemicals can utilize the ability to search for data from similar compounds, use the literature search and chemical predictions features, link to other applications, as well as potentially find in vitro data. Finally, the stakeholder with several chemicals can perform a batch search and download relevant information in one file. The interconnectedness of the EPA's CompTox Chemicals Dashboard allows you to discover the breadth of information available for your chemical

of interest. This talk will describe the data in and links from the Dashboard, and provide case examples for stakeholders, similar to those above, on how to navigate as well as use the data. *The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the US EPA.*

**8.05A.T-03 EAS-E Suite: A data integration and modelling framework to improve the understanding of chemical exposures and effects on humans and the environment.**

***Jon A. Arnot***[1,2]*, Alessandro Sangion*[1,2]*, Li Li*[3]*, Trevor N Brown*[1,4]*, Liisa Toose*[1]*, Alexandra Looky*[1]*, Lauren Hughes*[1] *and James M. Armitage*[5]*, (1) ARC Arnot Research & Consulting Inc., Canada, (2) University of Toronto Scarborough, Canada, (3) University of Nevada, Reno, (4) TNB Research, Malaysia, (5) AES Armitage Environmental Sciences, Inc, Canada*

Many data sources are required to conduct chemical assessments including use information, chemical properties, system and receptor information, toxicokinetics (TK), as well as toxicity data. Data and tools to improve the understanding of chemical exposures and effects on humans and the environment are required and dispersed and compiling and harnessing this information is challenging. The Exposure And Safety Estimation (EAS-E) Suite is a freely accessible on-line platform that addresses many challenges for ecological and human health assessment and facilitates the safe and sustainable production and use of chemicals in society. EAS-E Suite is comprised of (i) chemical information databases (e.g., physical-chemical properties, in vitro and in vivo toxicokinetics data, production volumes), (ii) quantitative structure-activity relationships (QSARs) for predicting chemical properties and half-lives, and (iii) various models and tools for chemical hazard, fate, TK, exposure, and risk estimation. EAS-E Suite includes (i) the CiP-CAFE model for chemical mode-of-entry and emission rates, (ii) the RAIDAR model for chemical fate in the natural environment and exposures to a range of ecological receptors, (iii) the RAIDAR-ICE model for indoor chemical fate and near-field human exposure simulations linking external and internal doses, and (iv) the PROTEX-HT model for aggregate human exposure and risk estimation. Other models in EAS-E Suite are (i) the in vitro mass balance model for simulating the fate of chemicals in in vitro bioassays, (ii) the EAS-E Suite and US EPA's high-throughput toxicokinetic models for forwards and reverse dosimetry in humans, rats, and fish, (iii) in vitro-in vivo extrapolation (IVIVE) models, (iv) various dermal exposure and (v) environmental fate models. EAS-E Suite accesses these databases and models with user supplied chemical name, CAS, or SMILES entry information. Furthermore, EAS-E Suite "autoparameterizes" the built-in tools and models for >70,000 discrete organic chemicals making it easy for the application of this information for experts and non-experts. The platform provides access to many useful databases and models as well as knowledge transfer to multiple stakeholders fostering collaboration and consensus building. This presentation summarizes key concepts of the EAS-E Suite platform showing how it integrates various data sources and model for chemical assessment and management objectives for new and existing chemicals.

**8.05A.T-04 A unified framework to enable streamlined RNA-seq analysis for non-model organisms**

*Peng Liu,* ***Jessica Ewald****, Orcun Hacariz, Eléna Legrand, Zhiqiang Pang, Guangyan Zhou, Jessica Head, Nil Basu and Jianguo Xia, McGill University, Canada*

Transcriptomics data promises to play a key role in the transition from traditional to alternative toxicity testing methods, however the use of RNA-seq data from non-model organisms is challenging due to its dependence on high-quality genome assembly and gene annotation. Such a framework, developed for model species in biomedical studies, is costly and unscalable for large-scale environmental toxicogenomics applications. Here, we introduce a new paradigm together with high-performance, user-friendly implementations to enable high-throughput RNAseq analysis in a species-independent manner. In this new proposed approach, transcript assembly and annotation are replaced with the alignment of individual reads directly to a large-scale, high-resolution ortholog database created from the genomes of hundreds of eukaryotic species. Downstream statistical and functional analysis is performed on an ortholog counts table rather than on a transcript counts table. The high-resolution ortholog database allows harmonization of gene symbols, descriptions, and gene set libraries across many species. These are all linked to publicly searchable ortholog IDs versus the ad hoc

annotation and ID systems typical of *de novo* assemblies. The software presented here (EcoOmicsAnalyst.ca, ExpressAnalyst.ca, and EcoOmicsDB.ca) that implement this conceptual framework enable RNA-seq profiling for any eukaryotic species under 24 hours on a regular laptop computer. The utility of this framework is demonstrated in a case study involving RNA-sequencing data from three salamander species, one with a reference genome and two without.

**8.05A.T-05 Functional Annotation of Genome Content via Conserved Protein Domain Architecture**
***Aron Marchler-Bauer**, National Library of Medicine*
In order to provide capabilities for comparative analysis of genomes and genome content, we are supporting efforts to increase the fraction of protein products that carry functional annotation and to standardize protein product names. To this end, we are curating names and functional descriptions for common conserved protein domain architectures as defined by the Conserved Domain Database (CDD). I will describe strategies to improve coverage and accuracy of annotation, and how domain architectures based on CDD are used in the functional annotation of prokaryotic genomes.

**8.05A.T-06 SeqAPASS implements strategic connections of knowledge streams to inform species extrapolation for chemical safety**
***Carlie LaLone**, U.S. Environmental Protection Agency*
No one tool or methodology can provide all necessary information regarding the impacts of a chemical to a species, let alone to the diversity of species. Transparent, scientifically supported development of tools that strategically connect to one another to fulfill data and knowledge needs for decision-making are promising for replacing and complementing toxicity testing. Therefore, throughout the tool development process, interoperability and connectivity to the correct resources is critical. The Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) tool was created to bring together tools and databases for the purpose of informing challenges in species extrapolation relevant to researchers and decision-makers concerned with chemical safety. The SeqAPASS tool specifically compares protein sequence and structure information across species to predict chemical susceptibility by querying a known sensitive species with the known chemical protein target and providing lines of evidence for structural conservation in other species. Connectivity with the National Center for Biotechnology Information and combination of their databases and executables make up the backend of the SeqAPASS tool. Linkages to DrugBank, the veterinary substances database, therapeutic target database, the toxin and toxin-target database, AOP-Wiki, and CompTox Chemicals Dashboard provide information needed for querying the SeqAPASS tool regarding sensitive species and chemical-protein targets. Autocompletion of a Boolean string to query Google Scholar from the SeqAPASS interface informs more advanced Levels of the SeqAPASS evaluation focusing on critical amino acid comparisons, whereas a widget to filter data from the ECOTOX knowledgebase provides opportunities to bring computational predictions of chemical susceptibility together with empirical data more rapidly. Ensuring the most useful information is available and linked to the user interface provides the ability to rapidly and systematically explore scientific questions and synthesize results as opposed to waste time searching for valuable information. To further advance the SeqAPASS pipeline, efforts are underway to incorporate tools for generating protein structural models, evaluating structural model quality, aligning structures, and generating additional metrics to improve understanding of protein conservation across species. This abstract neither constitutes nor necessarily reflects USEPA policy.

**8.05A.T-07 US-align: Universal Structure Alignments of Proteins, Nucleic Acids, and Macromolecular Complexes**
***Chengxin Zhang**[1], Morgan Shine[1], Anna M. Pyle[1] and Yang Zhang[2], (1) Yale University, (2) University of Michigan*
Structural comparisons and alignments are of fundamental importance for nearly all aspects of structural biology studies, ranging from structure family classification and structure-based function annotation to rational

protein design and drug discovery. Due to the distinct natures of different molecules, nearly all existing structure comparison algorithms have been developed for performing alignments on specific molecule types and operations; this can impede the assessments and function annotations of inter-molecular complexes across different molecule types, such as protein-protein and protein-nucleic acids interactions, although cellular life processes often involve such important complex interactions. In this work, we proposed the first type of unified structural alignment algorithm, US-align (https://zhanggroup.org/US-align/), which allows for universal structure comparisons from proteins, RNAs, and DNAs, either on monomer or complex form and including both pairwise and multiple structure alignments. Due to the extensive optimization of a uniform TM-score objective function powered by efficient heuristic search algorithms, the US-align method is around 10-100 times faster and yet generates more accurate alignments compared to the state-of-the-art individual methods that were developed to perform specific molecular structure alignments. This study demonstrates the technical advantage of integrating the structural alignments of different macromolecules into a unified approach. Meanwhile, the simplicity and facilitation of an effective universal algorithm will significantly improve the practical usefulness to biological users.

**8.05A.T-08 Enhancing the FAIRness (Findability, Accessibility, Interoperability, Reusability) of the AOP-Wiki**

***Stephen Edwards**[1], Ginnie Hench[1], Travis Karschnik[2], Daniel L. Villeneuve[3], Jason O'Brien[4] and Clemens Wittwehr[5], (1) RTI International, (2) General Dynamics Information Technology, (3) U.S. Environmental Protection Agency, (4) Environment and Climate Change Canada, (5) European Commission's Joint Research Centre, Italy*

The AOP-Wiki provides a central repository for the development of adverse outcome pathways (AOPs) and the dissemination of AOP information without restrictions. Each AOP is described in narrative form with interconnected pages that house information on the key events (KEs) and key event relationships (KERs) that make up the AOP. The KE pages contain information regarding the measurement of biological phenotypes indicative of toxicity progression from perturbation of the molecular initiating event to manifestation of an adverse outcome. The KER pages document the evidence supporting a causal relationship between the key events and can include quantitative considerations when available. Expanding the description of these elements with ontology-based expressions improves *findability* while also increasing the number of computational applications. The AOP-Wiki currently houses almost 400 AOPs containing over 1,500 KEs and 2,000 KERs. This information is available via a web interface for browsing and searching content and is also available for download (XML formatted files) or via an application programming interface (JSON or XML formats) to support the development of third-party tools or provide real-time data for computational analyses. This presentation will highlight recent updates to the AOP-Wiki designed to make the information in the wiki more *accessible* and *interoperable* both interactively and programmatically. This includes better use of biological ontologies to describe key events and more accurate representation of information regarding prototypical stressors, domains of applicability, and modulating factors. These enhancements also allow better documentation of the AOP development strategy to make the provenance of the knowledge more visible, which has been identified as a key requirement for engendering trust. A preview of upcoming features will include new visualization options and improvements to the underlying data model to promote crowdsourcing of AOP development and further improve transparency regarding the knowledge provenance. Finally, an overview of the *reuse* of information from the AOP-Wiki by third-party tools will demonstrate the added value that results from making the information freely available in a computable form. In some cases, the third-party tools have further improved the FAIRness of the wiki data. [The contents of this abstract neither constitute nor necessarily reflect official policies of the authors' organizations.]

**8.05A.T-09 Geospatial Analyses and Applications in Support of Integrative Environmental Health Science**

***Daniel K. Jones**[1], Stephanie E. Gordon[1], Brianna Williams[1], Annie Putman[1], Molly Blakowski[2] and Matthew*

*Morriss[1], (1) U.S. Geological Survey, (2) Utah State University*
Complex environmental health questions require a multidisciplinary integrative science approach to encompass the myriad of data sources, models, and endmembers. However, field and laboratory data are often collected and analyzed to address specific questions that may not readily integrate with other datasets and analytical approaches. The USGS Geospatial Analyses and Applications Laboratory (GAAL) maintains an extensive relational database of monitoring data and landscape characteristics, coupled with a suite of analytical tools to support rapid environmental health assessments. A foundational component of the GAAL's workflow is to work with environmental health scientists to 1) establish the key scientific questions, 2) identify data required to address the identified questions, 3) compile, format, and relate identified data to available monitoring locations, and 4) develop relational models that leverage geostatistical relationships between the monitoring and landscape data in support of the science questions. Through iterative development, the database and tools have been broadly applied to investigate a variety of environmental health questions including predicting endocrine disruption in the Chesapeake Bay Watershed in the eastern United States, evaluating risks posed by dust exposure in the Salt Lake Valley, Utah, and tracking contamination threats to drinking water. This talk will showcase several of the projects supported by GAAL with an emphasis on fostering new collaborative opportunities across the environmental health community.

**8.05A.T-010 Discussion**

**8.06.P Poster Only: Systems Approaches**

**8.06.P-We204 Use of avian indicators in evaluating remediation and restoration effectiveness**
***Daniel J. Sullivan**, Joseph Corra, Sophia Green, Tom Hollenhorst, Michael Kravitz, James M. Lazorchak and Marc Mills, U.S. Environmental Protection Agency*
The structure and function of riparian avian communities can be negatively influenced by anthropogenic stressors that affect the quality of the shoreline habitat or the nearby waterbody. Because avian populations often respond to changes in water quality and habitat, avian communities and use of the habitat may indicate when there is a change in environmental condition (e.g., habitat restoration, contaminant remediation) and provided ecosystem services. We are developing a monitoring program to evaluate responses of avian communities to remediation and restoration practices in two sites within Great Lakes Areas of Concern (AOCs): Cuyahoga River (Ohio) and St. Louis River Estuary (Wisconsin). Prior to remediation or restoration activities at these sites, we piloted the use of autonomous recording units (ARUs) and point-count surveys to assess avian community composition and what avian species were present with consideration to diet type (e.g., insectivores, piscivores), life history traits (e.g., wading, marsh species), etc. Here, we present how these findings will be used to assess; 1) the impact of future changes in water quality and habitat, and 2) anticipated linkages between avian communities, ecological condition (e.g., changes in habitat, water chemistry, contaminants, and food web structure) and ecosystem services associated with evaluating remediation and restoration effectiveness. Additionally, we will discuss potential differences in sampling methods between passive (ARU) and active (point-count) surveys and how each approach will be used to assess structure and function of avian populations and communities.

**8.06.P-We205 Evaluating Impacts to the U.S. Department of Defense Mission from Chemical Regulation of Phosphoric Acid, Triphenyl Ester (TPP) and Tris(2-chloroethyl) Phosphate (TCEP)**
***Kelsey Hendrixson**, Defense, Noblis, Inc.*
The U.S. Department of Defense (DoD) Chemical and Material Risk Management (CMRM) Program uses a three-tiered process for identification, assessment, and management of emerging chemicals. The CMRM Program identified two emerging chemicals and conducted an assessment to examine impacts to critical DoD functions and develop management options to address identified risks. This assessment qualitatively assessed the risks to DoD and the defense industrial base (DIB) from potential increased regulation of two phosphate

ester flame retardants (FRs), Phosphoric Acid, Triphenyl Ester (TPP) and Tris(2-chloroethyl) Phosphate (TCEP), under the U.S. Toxic Substances Control Act (TSCA) and the European Union's Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation. The assessment includes identifying the regulatory driver(s), gathering chemical usage data, and assessing potential impacts to the DoD.

The primary challenge in conducting the assessment involved collecting chemical and material (C/M) usage and application information for products and materials used by DoD and DIB. Tracking C/M content required identification of data for hardware and consumable uses. To address these challenges, search terms were used to query DoD data systems to locate consumable and hardware product use in weapon systems. Only chemical names and Chemical Abstracts Service Registry Numbers (CASRNs) were used as the primary search terms, and three properties of TPP and TCEP were used as secondary search terms.

TPP and TCEP are commonly used for their flame retardant properties in paints, coatings, sealants, rubber, plastic, and polymers. DoD uses TCEP and TPP in products such as hydraulic fluids, oils and greases, and preservative and sealing compounds. Additionally, phosphate ester FRs are known to be used as secondary plasticizers in polyvinyl chloride (PVC) plastics and polyurethane foams, however, the C/M data did not highlight any plasticizer applications. This may be a potential gap in the assessment of DoD's use of TPP and TCEP. Results presented include an assessment of potential impacts and recommendations for management actions. Relevance of this assessment extends beyond FRs in that the assessment tools can help improve DoD's ability to collect C/M content and provide better visibility into the DIB supply chain.

## 8.07.P Late Breaking Science: Systems Approaches

### 8.07.P-Mo202 Biopolymer-Based Food Packaging: A Green Approach Towards Sustainability

***Sneh Bangar*** *and William Scott Whiteside, Clemson University*

Globally, humans have generated 8.3 billion tons of plastic to date, and more than 90% of plastic is not recycled and goes to landfills or natural environments. Around 40% of plastic produced is packaging, used just once, and then discarded. Therefore, there is a need to develop alternative packing materials which are effective, biodegradable, and environment friendly. Biopolymers, including starch, have been explored as potential alternatives to conventional plastic packaging materials. However, poor processibility, restricted tensile strength, and high-water absorption capacity of starch films limit their practical applications. Cellulose nanocrystals (CNCs) extracted from plant tissues have shown promise to improve the properties of biopolymers by improving mechanical, barrier, and thermal properties. Reinforcement of Kudzu CNCs in starch-based films offers a promising candidate for developing biodegradable films with enhanced properties. Our research aimed to develop pearl millet starch-based bioplastic films reinforced with kudzu CNCs as an alternative to plastic food packaging. Results demonstrated that incorporation of kudzu CNCs improved crystallinity, heat and water-barrier properties, tensile strength, and Young's modulus of nanocomposite films, with optimum properties achieved at 5% CNCs. Further, the biopolymer films were subjected to shelf-life analysis and biodegradability tests. Biopolymer films successfully maintained the quality of fresh red grapes for up to 15 days of storage at 5°C. Also, the films were completely degraded within 21 days of the soil burial test. Our results suggest that biopolymer-based films have a great potential to be used as a sustainable packaging material for the short-term storage of foods.

### 8.07.P-Mo203 A cradle-to-gate life cycle assessment of second-generation biomethane production in Europe: A comparative review

***Taiwo Olanrewaju Omotosho***

A global challenge about resource strategical approach and the requirement to reduce the environmental impacts of fossil fuels production have soared the utilization of renewable energy sources like biomethane. Biomethane can be produced from upgraded biogas through anaerobic digestion in a popular technological process that satisfies the demands placed on it by the law, agronomy, and the environment for the development of rural economies and the generation of sustainable biofuels. In Europe, a relatively low research on upgrading biogas from second generation biomass (SGB) has been carried out in the last few years looking at sustainability and impact from life cycle perspective. The aim of this paper is to evaluate the state of biomethane production and the environmental impact as a result of greenhouse gases (GHG) and other environmental factors from SGB. This study reviewed and critically examined the reported literature of 15 life cycle assessment (LCA) studies of biomethane systems from SGB around Europe. More than 90% of the papers that have been reviewed demonstrate a decrease in global warming potential GWP, and in the same vein, the impact categories of acidification and eutrophication formation have decreased as a result of the availability of feedstock and the use of biogas digestate as manure in stead of mineral fertilizer. This increased carbon sequestration to the soil. Comparing the LCA outcomes was fairly challenging due to fewer studies showing inconsistency as a result of default databases from decision support tools used, however the review found that the system boundary, functional unit, and other LCA methodological elements have an impact on the studies' end results. We discovered there are a few studies on carbon capturing and storage CSS as upgrading technology to enhance carbon mineralization that prevents methane ($CH_4$) and carbo dioxide ($CO_2$) leaks as neutral carbon. However, despite a few LCA studies on the production of biomethane in Europe, these papers currently pay little attention to uncertainty analysis in their studies.

### 8.07.P-Mo204 Time-dependent Assessment of Embodied Carbon of Harvested Wood Products in California

***Poonam Khatri****, Prakash Nepal, Richard Bergman, David Nicholls, Kamalakanta Sahoo and Andrew Gray, U.S. Department of Agriculture*

Embodied carbon has become a common environmental metric to compare different products, especially in the construction industry. The estimation of harvested wood products (HWPs) embodied carbon in this study for the State of California was based on life cycle assessment (LCA) methodology including all life stages from cradle-to-grave. In conventional LCA, all GHG emissions are aggregated and considered to occur at year 0 of the time horizon (usually 100 years). In this study, a dynamic LCA methodology was applied to five HWP classes (softwood lumber, non-structural panels, softwood plywood, other industrial products, and softwood utility poles). Dynamic LCA considers timing of GHG emissions, and their warming effect was determined using time-dependent characterization factor over a longer time horizon (500 years). A considerable difference was noticed in the results of two approaches: static and dynamic LCA. The choice of LCA approach could significantly influence the estimation of substitution benefits when HWPs are compared with fossil-intensive non-wood products. In addition, the choice for end-of-life disposal of HWPs influenced dynamic LCA results. Overall, the dynamic LCA performed in this study enabled more robust interpretations of embodied carbon by including temporal boundaries. A yearly profile of the warming effect of GHGs for different HWPs and end-of-life scenarios would help decision makers make timely decisions to reduce long-term GHG emissions.

### 8.07.P-Mo205 The Ebbs and Flows of Piloting SARS-CoV-2 Wastewater Sequencing in Texas

***Bonnie Oh****[1], Karen Bobier[1], Mayela Pedrueza[1], Michael Jost[1], Laura M. Langan[2], Bryan W. Brooks[2], Jessica Labonte[3], Yan Sun[1] and Rachel Lee[1], (1) Texas Department of State Health Services, (2) Baylor University, (3) Texas A&M University at Galveston*

With the SARS-CoV-2 Pandemic surging around the globe, institutions began focusing more on wastewater sequencing to potentially predict clinical trends in variants and outbreaks. In partnership with Baylor University and Texas A&M University, the Texas Department of State Health Services (DSHS) Laboratory participated in an FDA Pilot Project focused on SARS-CoV-2 sequencing of wastewater influent from two neighboring poultry packing plants. Samples were collected twice a week from January to August 2022 from manholes on a public easement to test only the influent from these two distinct factories. Choosing collection sites close to food manufacturing sites provided a unique focus on small populations (<1000 people) with direct contact to manufacturing food sources, as most other influents tested come from large populations or residential areas. Samples were concentrated by centrifugation, extracted, and quantitative Polymerase Chain Reaction (qPCR) performed to detect the Nucleocapsid (N1) gene. Reverse-transcription PCR and Tiling PCR were performed on positive samples and library preparation was completed for amplicon-based sequencing on Illumina MiSeq instruments. Sequences were analyzed using Texas DSHS's in-house Cecret bioinformatics pipeline for quality, genome coverage, and lineage identification. A total of 131 samples were collected from these sites, 26 were qPCR positive for SARS-CoV-2, and 12 had acceptable coverage and read quality to be submitted to NCBI's sequence read archive. The predominant lineage proportions identified over time were compared to the Omicron variant trend seen from clinical SARS-CoV-2 sequencing data. Challenges and successes regarding methods for extraction, purification, cDNA generation, sequencing, and analysis will be presented. Next steps for the project are to continue optimizing sample preparation and sequencing methods, while looking into other sites with high public health impact, such as nursing homes and correctional facilities to help stem the tide of future outbreaks for the State of Texas.

## 8.07.V Late Breaking Science: Systems Approaches

### 8.07.V-01 Modernization of Chemical Hazard and Safety Data Management Systems

***Kevin Patrick Fenton***[1]*, Steve Simske*[1] *and Jonathan Luu*[2]*, (1) Colorado State University, (2) United States Air Force*

Chemical hazards for human health and the environment are primarily communicated to the public through Safety Data Sheets (SDSs) from the chemical vendors or distributors. Despite the criticality for hazard communication precision, workplace hazard communication violations remain among the top citations during EHS inspections by regulatory agencies. Incorrect SDSs can yield significant liability as subsequent environmental and occupational health analyses and reporting are based upon incorrect and, in some cases, entirely different chemical formulations.

Our research focuses on the need for a paradigm shift in our chemical management systems and how a standardized management system and various recent technological advances can be incorporated into chemical lifecycle processes to reduce or eliminate these liabilities. The following advancements and strategies were researched as ways to enhance the communication and data management of workplace chemicals, reduce potential exposure and spill risks, reduce workplace hazards, and increase the efficiency and accuracy of environmental reporting through a more streamlined systems approach:

-The need for a centralized universal SDS repository with full chemical disclosure of all product constituents and a nationally adopted machine language SDS standard

-The use of Artificial Intelligence in EHS Systems, specifically, how they can be used as a medium to transition towards an automated standard by reverse-engineering and partitioning SDS components into machine-encoded text that can be validated and uploaded to a centralized repository or downstream databases

-Algorithmic and Meta-algorithmic approaches to SDS requirement and data validation, hazard characteristic code calculations, and in determination of potentially less hazardous substitutions

-Application of Natural Language Processing (NLP) methods for real-time updates from scientific journals, regulatory agencies, etc. to produce "living" SDSs capable of informing users of relevant regulatory updates, research, etc.

-Embedded SDSs or SDS links in product barcodes with QR Code reader technology to retrieve precise SDSs for each product for instant and precise retrieval abilities in emergency situations

-Benefits of RFID technology in providing accurate SDS associations while also minimizing manual tracking of hazardous material and waste containers and monitor for expired shelf life, incompatible storage, temperature sensitivities, and other inventory concerns

**8.07.V-02 A Systems-Toxicology Perspective of Critical Metals Recovery From Waste Printed Circuit Boards: Opportunities, Risks, and Barriers for a Circular Supply Chain**

***Maryam Gamal Ibrahim**, Julie M Schoenung and Oladele Ogunseitan, University of California, Irvine*

The strategic use of critical metals in the development of advanced materials and multifunctional components in products has opened new avenues for innovative, emerging technologies. Currently, the electronics industry is at the forefront of rapid technological developments that have caused a digital shift in all areas of industry and a growing consumer demand for high-tech devices. However, the global supply of critical metals is impacted by geo-political barriers, declining ore grades, and price volatility which have caused shortages and disruptions. At present, the electronics industry is the largest consumer of critical metals, for which global resource demand is projected to double by 2030. As traditional mining becomes unsustainable, the need to shift to a circular economy through the urban mining of rich secondary sources (such as e-waste) becomes an economic priority and a national security incentive to meet future resource demands and secure the stability of industries. President Biden's recent executive order (E.O. 14017, 2021) emphasized the urgency of securing resilient U.S. critical metals supply chains. Waste printed circuit boards (WPCBs) contain the widest variety and highest concentration of critical metals than any other component of the e-waste stream. Recycling WPCBs provides high economic value and an opportunity for improving resource management, however, the presence of highly toxic substances in WPCB materials presents major challenges for resource recovery. In the current baseline scenario for end-of-life electronics, the accumulation of global e-waste in developing countries has led to a public health crisis for vulnerable communities, significant environmental degradation, and a waste of valuable resources. This research explores the potential for establishing a domestic circular supply chain for recovering critical metals from WPCBs by identifying the current risks and barriers from a systems-level perspective of human and environmental health. Here, the sustainability of current domestic recycling technologies is evaluated for the pre-processing, extraction, and purification of critical metals from WPCBs. The technological and environmental barriers of implementation are also analyzed and discussed to identify gaps in hazard control, resource efficiency, and economic feasibility. The results of this work will inform regulatory strategies and collaborative efforts to support a circular supply chain of critical metals from WPCBs.

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