ABSTRACT BOOK

Society of Environmental Toxicology and Chemistry North America 42nd Annual Meeting – SETAC SciCon$_4$

Solutions With Respect for Our Community and Environment

14–18 November 2021 • Virtual

Abstract Book

SETAC North America 42nd Annual Meeting

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This book comprises the abstracts of the presentations for the platform and poster sessions of the Society of Environmental Toxicology and Chemistry (SETAC) North America 42nd Annual Meeting, conducted virtually from 14–18 November 2021. The abstracts are reproduced as accepted by the Scientific Program Committee and appear in numerical order.

In each abstract, the presenting author's name is underlined. The author index cross-references the corresponding abstract numbers.

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About SETAC

The Society of Environmental Toxicology and Chemistry (SETAC), with offices in North America and Europe, is a non-profit, 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

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

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

SETAC membership currently comprises about 4,500 individuals from government, academia, business and nongovernmental organizations with backgrounds in chemistry, toxicology, biology, ecology, atmospheric sciences, health sciences, earth sciences, environmental engineering, hazard and risk assessment, and life cycle assessment.

If you have training in these or related disciplines and are engaged in the study, use or management of environmental resources, SETAC can fulfill your professional affiliation needs.

All members receive the SETAC Globe newsletter highlighting environmental topics and SETAC activities, reduced fees for meetings and discounts on SETAC books. All members receive online access to *Environmental Toxicology and Chemistry* (ET&C) and *Integrated Environmental Assessment and Management* (IEAM), the peer-reviewed journals of the society. Members may hold office and, with the Emeritus Members, constitute the voting membership.

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Environmental Quality Through Science®

Solutions With Respect for Our Community and Environment

The theme of the SETAC North America 42nd Annual Meeting – Solutions With Respect for Our Community and Environment – was selected late in 2020 at the height of the pandemic to play on the idea of our participants coming together to collaborate in community as we had really hoped for an in-person gathering in 2021. The theme was a nod to what was expected to be a change in direction from governments around the world that would be more aligned with the vision of our society, which is Environmental Quality Through Science®. The scientific program was organized into eight tracks:

Environmental Toxicology and Stress Response

Explores environmental toxicology and response to stress (biological, physical and chemical) in various system. Encompasses in silico and in vitro tools and methods involving adverse outcome pathways (AOPs), mode of action, molecular toxicology, -omics, animal alternative testing, quantitative structural activity relationships (QSARs), high-throughput techniques and emerging approaches for statistical toxicology.

Aquatic Toxicology, Ecology and Stress Response

Explores ecology, ecotoxicology and response to stress of all aquatic systems, including lentic and lotic freshwater systems, estuaries, coastal and marine environments.

Wildlife Toxicology, Ecology and Stress Response

Covers all life forms of wildlife not strictly aquatic (amphibian, reptile, birds and mammals and other organisms) living in areas from the deserts to the tropics and everything in between.

Environmental Risk Assessment

Bridges both aquatic and terrestrial environments, and all potential stressors (physical, chemical, biological and biotechnological) with human and ecological endpoints towards the goal of integrated holistic assessment such as “One Health.”

Policy, Management and Communication

Includes all aspects of science application in policy or regulations and management (regulatory science), as well as science commination to stakeholders in diverse audiences.

Engineering, Remediation and Restoration

Addresses remediation and restoration of stressor impacted air, water, and soil and sediment, including tools for predicting, monitoring and evaluation; technologies and methods for remediation and restoration; environmental engineering; green remediation; damage assessment; and strategies for management.

Chemistry and Exposure Assessment

Comprises all aspects of chemical analysis, monitoring, fate and modeling, green chemistry and alternative chemical assessment.

Systems Approaches

Uses cross- and trans-disciplinary approaches seeking to address complexity and large-scale issues by applying and integrating concepts such as life cycle assessment, sustainability, ecosystem services, impact assessment and environmental economics. Topics include regional and watershed-scale environmental management, climate change, resiliency and other related areas.

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Daily Schedule

Sunday, 14 November

TIME (PST)	NETWORKING EVENTS
14:00–15:00	SETAC Meet and Greet
15:00–17:00	Virtual Student Corner

Monday, 15 November

TIME (PST)	NETWORKING, PLENARIES, TRAINING	
7:00–8:00	Buddy Program Meet and Greet	
8:00–9:00	Plenary: Maia D. Bellon: Intersectional Environmentalism	
9:00–9:30	Break	
9:30–11:30	On-Demand Presentations Presenters Available for Synchronous Q&A	Exhibitors Meet and Greet
11:30–12:30	Peer-Review Training and Networking	
12:30–13:00	Break	

TIME (PST)	SESSION DISCUSSIONS				
13:00–15:00	Background: Soil, Sediment, Water, Air	Where the Tire Rubber Meets the Road	Alternative Approaches to Animal Testing	Pelagic and Benthic Harmful Algal Blooms	Fate and Effects of Metals: Bioavailability and Toxicity

TIME (PST)	TOPICAL MIXERS				
15:00–16:30	Land Back and Indigenous Science	Tire Wear Particles Test Material		Casual Networking	Metals
16:30–18:30	Alumni				

Daily Schedule

Tuesday, 16 November

TIME (PDT)	DAILY PLENARY
8:00–9:00	Plenary: Vikas Sharma: Data and Algorithms Help Leaders Solve Problems
9:00–9:30	Break

<table>
<tr><th>TIME (PDT)</th><th colspan="5">SESSION DISCUSSIONS</th></tr>
<tr><td>9:30–10:30</td><td rowspan="2">Multiple Stressors and Mixtures</td><td rowspan="2">Microplastics: Standards, Big Data and Analytica; Methods</td><td rowspan="2">Bioaccumulation: Linking Exposure to Effects in Aquatic Ecosystems</td><td>Advancing Use of Behavioral Studies</td><td rowspan="2">Wildlife Ecotoxicology: Conservation and Regulatory Connections</td></tr>
<tr><td>10:30–11:30</td><td>Pharmaceuticals in the Environment</td></tr>
</table>

TIME (PDT)	SPECIAL SESSION
9:30–11:30	Environmental Justice

TIME (PDT)	NETWORKING, PLENARIES, TRAINING		
11:30–13:00	Women in SETAC	Agilent Expert Seminar	Syngenta Expert Seminar

<table>
<tr><th>TIME (PDT)</th><th colspan="5">SESSION DISCUSSIONS</th></tr>
<tr><td>13:00–14:00</td><td rowspan="2">Lab and Field Collected Invertebrates</td><td rowspan="2">Canada’s Oil Sands and Dilbit</td><td>New Approach Methodologies- From Research to Risk Assessment</td><td rowspan="2">Effects of Early Life Exposure to Contaminants in Fish</td><td rowspan="2">Wildlife Ecotoxicology: Exposure and Effects</td></tr>
<tr><td>14:00–15:00</td><td>Passive Sampling: Recent Advances and Solutions</td></tr>
</table>

TIME (PDT)	SPECIAL SESSION
13:00–14:00	Regulatory Update: Metals in the Environment

<table>
<tr><th>TIME (PDT)</th><th colspan="5">TOPICAL MIXERS, NETWORKING</th></tr>
<tr><td>15:00–16:30</td><td>Chemistry Interest Group</td><td>Beer Nirvana</td><td>Science and Risk Communication (SCIRIC)</td><td></td><td></td></tr>
<tr><td>16:30–18:00</td><td colspan="5">Early Career Social</td></tr>
</table>

Wednesday, 17 November

TIME (PST)	DAILY PLENARY
8:00–9:00	Plenary: Kate Moore: The Radium Girls
9:00–9:30	Break

<table>
<tr><th>TIME (PST)</th><th colspan="5">SESSION DISCUSSIONS</th></tr>
<tr><td>9:30–10:30</td><td rowspan="2">Puget Sound</td><td>Microplastic Monitoring and Risk Characterization for Management Strategies</td><td rowspan="2">New Approach Methodology and the Endocrine Pathway</td><td rowspan="2">Immunotoxicology</td><td>Complex Mixture Ecotoxicity</td></tr>
<tr><td>10:30–11:30</td><td>Microplastics Research Priorities: Detection, Analysis and Effects</td><td>Engineering, Remediation and Restoration</td></tr>
</table>

TIME (PST)	SPECIAL SESSION
9:30–11:30	Integrating Emerging Science in Wildlife Chemical Risk Assessment

TIME (PST)	STUDENT SEMINAR
11:30–13:00	Robyn Leigh Tanguay: Thinking Outside the Tank to Protect the Environment and Human Health

<table>
<tr><th>TIME (PST)</th><th colspan="5">SESSION DISCUSSIONS</th></tr>
<tr><td>13:00–14:00</td><td>Portland Harbor</td><td rowspan="2">Trends in Environmental Risk Assessment of Pesticides</td><td>Adverse Outcome Pathways</td><td>Fate and Effects of Chemicals from Stormwater Runoff</td><td>Chemicals in Arctic and Antarctic Environments</td></tr>
<tr><td>14:00–15:00</td><td>Understanding Diversity in Species Sensitivity</td><td>Environmental Fate and Effects of Nanomaterials</td><td>Non-Target Analysis: Prioritization of Organic Contaminants</td><td>Plant Ecotoxicology and Risk Assessment</td></tr>
</table>

TIME (PST)	SPECIAL SESSION
13:00–14:00	Sustainability in Indigenous Food Systems

<table>
<tr><th>TIME (PST)</th><th colspan="5">TOPICAL MIXERS, NETWORKING</th></tr>
<tr><td>15:00–16:30</td><td></td><td>Microplastics Interest Group</td><td></td><td></td><td>Plants</td></tr>
<tr><td>16:30–17:00</td><td colspan="2" rowspan="2">Inclusive Diversity Committee GEM Social</td><td colspan="3"></td></tr>
<tr><td>17:00–19:00</td><td colspan="3">Massive Team Trivia Student Mixer</td></tr>
</table>

Daily Schedule

Thursday, 18 November

TIME (PDT)	ASSEMBLY
8:00–9:00	SETAC North America General Assembly
9:00–9:30	Break

TIME (PDT)	SESSION DISCUSSIONS				
9:30–11:30	When Standardized Regulatory Tests Are Not Enough	Contaminants of Emerging Concern and Superfund	Adapting Toxicity Test Methods to Atypical Sample Types	Alternatives Assessment and Informed Substitution	Bioinformatics in Cross-Species Extrapolations

TIME (PDT)	SPECIAL SESSION
9:30–10:30	Teaching Environmental Toxicology and Chemistry During and After COVID-19

TIME (PDT)	
11:30–13:00	Break

<table>
<tr><th>TIME (PDT)</th><th colspan="5">SESSION DISCUSSIONS</th></tr>
<tr><td>13:00–14:00</td><td rowspan="2">Endangered Species Risk Assessment of Pesticides</td><td rowspan="2">Microplastics and Nanoplastics: Fate, Effects, Risk, and Management</td><td>Importance of Science-Based Research</td><td rowspan="2">Elucidating Chemical Transformations in Environmental Fate Research</td><td rowspan="2">Developing a STEAM Outreach Program</td></tr>
<tr><td>14:00–15:00</td><td>Science-based Consensus</td></tr>
</table>

TIME (PDT)	CLOSING
15:00–16:00	Closing Remarks

Abstracts

01.01 Advancing the Use of Existing Data in Ecotoxicology

01.01.01 The ECOTOXicology Knowledgebase Literature Search and Review Processes for Identifying and Curating Toxicity Data for Ecological Risk Assessments

J.H. Olker, U.S. Environmental Protection Agency / Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division; C. Elonen, U.S. Environmental Protection Agency / Office of Research and Development, Center for Computational Toxicology and Exposure; A. Anderson, K. Nehiba, T. Scott, T. Karschnik, A. Pilli, General Dynamics Information Technology; D.J. Hoff, U.S. Environmental Protection Agency / Office of Research and Development

The need for assembled existing toxicity data has accelerated as the number of chemicals introduced into commerce continues to grow. To address this evolving need, the ECOTOXicology Knowledgebase (ECOTOX) has been locating and curating ecologically-relevant toxicity data for over 30 years and is now a nationally and internationally recognized source of single-chemical toxicity test results for aquatic and terrestrial organisms. ECOTOX currently includes data for over 12,000 chemicals and 13,000 ecological species, with over one million records from over 52,000 references and quarterly updates to public website (www.epa.gov/ecotox). Presented here is an overview of ECOTOX, detailing the systematic and transparent literature review and data curation processes, including how the well-established protocols align with current systematic review and evidence mapping practices, and the recent incorporation of data analytics tools to improve efficiency and transparency of the pipeline. ECOTOX conducts chemical-based comprehensive literature searches and screens titles and abstracts to identify references that meet applicability and initial acceptance criteria (e.g., ecologically-relevant species, verifiable CASRN, endpoint and control reported). Acceptable studies are identified with full-text screening and all pertinent study and effects information (e.g., species, chemicals, test methods and conditions, toxicity results) are extracted following controlled vocabularies. ECOTOX is utilized by government entities, academia, and industry for ecological risk assessment, chemical prioritization, emergency response, and development of models, tools, and applications (e.g., QSARs, Species Sensitivity Distributions, Adverse Outcome Pathways). Recent enhancements to ECOTOX include an updated user interface, interactive queries and data visualization options, and incorporation of standard species and chemical identifiers, with on-going efforts to harmonize terminologies with existing ontologies. ECOTOX continues to be a flexible resource to help meet the challenge of providing empirical toxicity data at a faster pace for chemical decision-making. The incorporation of more efficient processes and the updated user interface provide the regulated industry and researchers more frequent data updates and improved methods to search and use existing toxic effects data to determine thresholds and conduct risk assessments. *This abstract does not necessarily reflect USEPA policy.*

01.01.03 Defining the Taxonomic Domain of Applicability of an Adverse Outcome Pathway Network Using Bioinformatics

M. Jensen, University of Minnesota-Duluth; D. Blatz, Oak Ridge Institute for Science & Education at USEPA / ORD/CCTE/Great Lakes Toxicology & Ecology Division; C. LaLone, U.S. Environmental Protection Agency / ORD/CCTE/Great Lakes Toxicology & Ecology Division

The adverse outcome pathway (AOP) framework captures and organizes existing biological pathway knowledge to direct research efforts and aid in risk assessment. This existing knowledge along with continuously expanding sequence data may also be used to define the taxonomic relevance of an AOP, which can support extrapolation from tested species to other non-tested species. Recently, an AOP network was developed linking activation of the nicotinic acetylcholine receptor to colony death/failure in honey bees (*Apis mellifera*). While there have been growing concerns regarding chemical stressors impacting honey bees, there is also concern for other pollinator species, including non-*Apis* bees. Evaluation of the potential adverse effects on pollinators caused by chemical and non-chemical stressors is crucial, as population declines could have significant economic and ecological consequences. Because AOPs are typically developed using existing knowledge from one, or handful, of species, there is a lack of knowledge regarding whether the pathways can be extrapolated to other untested species. To lay the foundation for defining the taxonomic domain of applicability (DOA) for this AOP network, which considers structural and functional conservation, the U.S. Environmental Protection Agency's Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) tool was used. Structural conservation of proteins identified in the key events and key event relationships of the AOP network were evaluated using SeqAPASS to understand how broadly the AOP could be extrapolated to other pollinator species. SeqAPASS evaluations revealed conservation across *Apis* and non-*Apis* bees at the molecular initiating event and early key events of the AOP network. For late key events, other species-specific factors can be considered to further define the DOA, such as differences in life-history, foraging activity, and colony size and structure. This work describes a path forward in incorporating bioinformatics to define the taxonomic DOA of an AOP, specifically focusing on adding lines of evidence for the structural conservation of key events and key event relationships. Additionally, defining the DOA for this AOP network provides insight relevant to filling research gaps and pesticide risk assessment for the protection of non-*Apis* bees and other pollinators. *The views expressed in this work are those of the authors and do not necessarily reflect the views or policies of the USEPA.*

01.01.04 Screening for Contaminants of Concern in Surface Water Using an Automated Exposure-Focused Workflow

J. Wall, U.S. Environmental Protection Agency / Center for Computational Toxicology & Exposure; C. Greene, Minnesota Department of Health; H. Goeden, Minnesota Department of Health / Environmental Health; A. Williams, U.S. Environmental Protection Agency / Center for Computational Toxicology & Exposure; J. Franzosa, U.S. Environmental Protection Agency / ORD/NHEERL; J. Wambaugh, U.S. Environmental Protection Agency / Center for Computational Toxicology and Exposure; A. Singh, U.S. Environmental Protection Agency / Center for Computational Toxicology & Exposure; M. Linnenbrink, U.S. Environmental Protection Agency / ORD; J.C. Lambert, U.S. Environmental Protection Agency / National Center for Environmental Assessment; K.K. Isaacs, U.S. Environmental Protection Agency / Center for Computational Toxicology & Exposure

Characterization of exposure risk for chemicals found in the environment is a task that often exceeds the capacity of government agencies due to the sheer number of chemicals found in the environment. Often, a manual screening process is performed using scientifically sound approaches to prioritize chemicals for further assessment. The Minnesota Department of Health (MDH), under its Contaminants of Emerging Concern (CEC) initiative, uses a standardized process to screen potential contaminants based on exposure potential. Recently, MDH partnered with the U.S. EPA Office of Research and Development (ORD) to accelerate this process via development of an automated workflow to collect and report relevant exposure data, including New Approach Methodologies (NAMs) for exposure from ORD's ExpoCast project and relevant chemical data found on ORD's CompTox Chemicals Dashboard. The workflow pulls from 23 broad data sources, covering five domains: chemical identity and use, properties, emission and disposal, environmental occurrence, and human exposure. The collected data were used to score chemicals on exposure potential and data availability using quantitative algorithms previously developed by MDH (score range of 0-10 for exposure potential; 0-5 for data availability). A validation test, comparing the output to 87 manually screened chemicals, confirmed agreement between the processes in most data domains. Here, the automated workflow was applied to a case study of 1,762 chemicals to identify potential candidates for the CEC program. The case study data was pulled from the U.S. EPA's

Multimedia Monitoring Database (MMDB) for all unique chemicals that were detected in surface water samples. The average time for a chemical to complete the workflow (pull relevant data, score, generate report) was approximately five minutes, with the quickest finishing in under two minutes and the longest taking two hours. This is an exponential increase in chemical screening rate compared to the manual process. Some higher-ranking chemicals (high adjusted exposure score and data availability) included Phenol (6.30; 3.81) and Acetophenone (5.21; 3.79). For reference, Lead (2.12; 1.60) and BPA (4.75; 3.48), both known chemicals of concern, scored lower. This case study provides an example of how promising automated workflows can be for accelerating chemical screening and prioritization processes by leveraging existing data structures and NAMs. This abstract does not reflect EPA policy.

01.01.05 Endogenous Lifecycle Models for Chemical Risk Assessment

M. Etterson, U.S. Environmental Protection Agency / ORD/CCTE/Great Lakes Toxicology & Ecology Division; G.T. Ankley, U.S. Environmental Protection Agency / Center for Computational Toxicology and Exposure

Despite over 50 years of research on the use of population models in chemical risk assessment, their practical utility has remained elusive. A novel application and interpretation of ecotoxicological models, Endogenous Lifecycle Models (ELM), is proposed that offers some of the benefits sought from population models, at much lower cost of design, parameterization, and verification. ELMs capture the endogenous lifecycle processes of growth, development, survival, and reproduction and integrate these to estimate and predict expected fitness. Two measures of fitness are proposed as natural model predictions in the context of chemical risk assessment, lifetime reproductive success, and the expected annual propagation of genetic descendants, including self (intrinsic fitness). Six characteristics of the ELM approach are reviewed and illustrated with two ELM examples, the first for a general passerine lifecycle and the second for Bald Eagle (*Haliaeetus leucocephalus*). Throughout, the focus is on development of robust qualitative model predictions that depend as little as possible on specific parameter values. Thus, ELMs sacrifice precision to optimize generality in understanding the effects of chemicals across the diversity of avian lifecycles. Notably, the ELM approach integrates naturally with the adverse outcome pathway framework; this integration can be employed as a mid-tier risk assessment tool when lower tier analyses suggest potential risk.

01.01.06 Using the ECOTOXicology Knowledgebase Protocols for Identification and Evidence Mapping of Ecological Toxicity Data for Per- and Polyfluoroalkyl Substances (PFAS)

D.J. Hoff, J. Olker, U.S. Environmental Protection Agency / Office of Research and Development; A. Pomplun, A. Anderson, K. Nehiba, General Dynamics Information Technology; D. Peterson, G. Elonen, SpecPro Professional Services; A. Pilli, General Dynamics Information Technology; C. Elonen, U.S. Environmental Protection Agency / Office of Research and Development, Center for Computational Toxicology and Exposure

The ECOTOXicology Knowledgebase (ECOTOX, www.epa.gov/ecotox) is the world's largest compilation of ecological toxicity data that has been curated over 30 years to support chemical assessments and research. Over the last three years, the well-established ECOTOX systematic and transparent procedures have been used to identify, assemble, and illustrate existing empirical toxicity data for per- and polyfluoroalkyl substances (PFAS). There is a recognized need to characterize potential ecological impacts of PFAS, however assessing the risks is challenging due to the large number of PFAS and ecological species potentially impacted. Thus, chemical assessments must initially rely on existing empirical data and computational models. Our goal was to identify and make readily available ecological effects data for PFAS through a) conducting comprehensive literature searches for over 400 PFAS names and CASRNs, b) reviewing the resulting list of over 160,000 references at the title and abstract level, c) evaluating acceptability for inclusion into ECOTOX at the full-text level and d) extraction of relevant information (species, chemicals, test methods, toxicity results) from all acceptable studies. To meet the challenge of an unprecedented level of information to gather and filter through, advanced data analytic tools were incorporated into the ECOTOX pipeline. This focused effort has resulted in the addition of over 700 references added to ECOTOX, which now includes over 25,000 test results from nearly 900 PFAS references. This total includes results from ~150 PFAS and over 450 biological species. Characterization of the extent, distribution, and types of evidence of the curated dataset to-date indicate that over 50 % of the data were from studies on PFOS and PFOA in about 14 species of fish, aquatic invertebrates, and birds. However, the diversity of chemicals and species represented has been expanding rapidly as we incorporate data from recently published studies. Traditional growth/reproduction/mortality toxicity endpoints represented about 25% of reported effects, while 34% were biochemical or genetic effects. This effort identified and curated relevant ecological toxicity data for PFAS suitable for ecological risk assessments and identified data gaps of biological species and classes of PFAS for prioritizing research needs. *This abstract does not necessarily reflect USEPA policy.*

01.01.07 Predicting Gastrointestinal Permeability of Organic Chemicals for Human Exposure and Risk Assessments

S. Wang, University of Nevada, Reno / Environmental Science; D. Li, L. Li, University of Nevada, Reno / Community Health Sciences

Ingested organic contaminants exert adverse health effects on humans after they are absorbed into the circulatory system and become "internal" doses. It is thus critical to mechanistically understand and defensibly quantify the permeability of the myriad of organic contaminants crossing various absorption barriers., e.g., gastrointestinal tract (GIT), blood-brain barrier, and skin. At the screening level, the gastrointestinal permeability is often experimentally determined by an in vitro approach called Parallel Artificial Membrane Permeability Assay (PAMPA). However, a single run of the PAMPA experiment can take anywhere from half an hour to 48 hours, which can be time-consuming and costly when facing the numerous amounts of organic contaminants of concern. In this presentation, we introduce an in-silico mass balance model in support of reliably predicting the effective permeability (P_e) for thousands of chemicals in seconds. The model mechanistically simulates the GIT-PAMPA experiment, based on inputs of only a few physicochemical properties, e. g., molecular weight (MW), octanal-water distribution coefficient (D_{OW}, for combined neutral and ionized molecular species), and hydrogen bonding properties. We test the model by reproducing 1500+ measurements collected from the National Center of Advancing Translational Sciences (NCATS) database. As our results show, 60% of the estimated P_e values fall into the same orders of magnitude as the experimental measurements, indicating the reliability of the developed model. The model results indicate that a chemical becomes highly permeable when log D_{OW} is between 2 and 4 and MW is less than 400, which agrees with the mechanistic process of passive transport through the lipid bilayer structure of cell membrane. The model is capable of providing academia and regulatory agencies with a worst-case scenario estimate of Pe (maximum possible Pe for any given log D_{OW} and MW) and thus a powerful tool in pre-screening thousands of commercial chemicals regarding their bioavailability through oral ingestion.

01.01.08 Estimating the Maximum Tolerated Concentration for Amphibian Metamorphosis Assays: Using Existing Toxicity Data to Guide Concentration Selection

J. Krzykwa, Smithers ERS, LLC / Environmental Toxicology; J. Schwalbe, Smithers ERS, LLC / Environmental Risk Sciences; J. Marini, Smithers Viscient / Aquatic Toxicology; L. Sayers, Smithers Viscient, LLC / Department of Ecotoxicology

Under current European Union regulations, all biocides and plant protection products must be evaluated for potential endocrine disrupting (ED) effects, specifically for estrogen, androgen, thyroid, or steroidogenic (EATS) modalities. The amphibian metamorphosis assay (AMA) is an *in* *vivo* assay with *Xenopus laevis* larvae used to screen for potential impacts on hypothalamus-pituitary-thyroid (HPT) signaling. The AMA is considered a level 3 Organisation for Economic Cooperation and Development (OECD) evaluation method in the OECD conceptual framework for the assessment of ED capabilities and is utilized to evaluate the potential for a compound to interact with the HPT axis. Such compounds may have acute/chronic fish data available from previous registration requirements, but there is rarely amphibian toxicity data. This lack of available amphibian toxicity data poses a challenge when selecting appropriate levels for ED testing, as the goal of ED testing is to test to levels high enough to investigate potential EATS activity without inducing overt toxicity, a level referred to as the maximum tolerated concentration (MTC). Current guidance in the OECD AMA guideline (OECD 231) suggests using 1/3 the LC50 value as a method for estimating the MTC; however, this method may not always be appropriate. The objective of this project was to provide guidance regarding the use of existing fish toxicity data for the selection of levels for the AMA; thus, reducing uncertainty around the identification of the MTC for testing and reducing the number of organisms needed for range finding studies. To achieve this goal, data from the ECOTOX database, the Office of Pesticide Programs, and the Screening Results and Data Evaluation Records for EDSP Tier I Chemicals was compared to evaluate how available acute/chronic fish data relates to the estimated MTC for AMAs. This meta-analysis will provide guidance for selecting levels during AMA testing; as well as potentially reducing the number of organisms needed during range finding studies for some well-described compounds.

01.01.09 Assessing Ecosystem Health Through Contamination in the Tijuana River Estuary

N. Torres, University of San Diego / Environmental and Ocean Sciences; J. Crooks, Tijuana River Estuary National Estuarine Research Reserve; C.Q. Zeeman, U.S. Fish and Wildlife Service / Environmental Contaminants Program; D. Talley, University of San Diego / Environmental and Ocean Sciences

Although the Tijuana River Estuary remains the largest, most-intact coastal wetland in Southern California, it has a history of major changes, much of this related to its location immediately north of the US / Mexico Border. One of the primary challenges is cross-border flows from the rapidly growing city of Tijuana, Baja California, and the delivery of wastewater, debris, and sediment to sensitive coastal wetland ecosystems. There is a need to more fully investigate these environmental changes to assess the ecosystem health of the Tijuana River Estuary over time, especially related to pollution impacts. This can inform an understanding of changes in both species and stressors, and can also help assess the effectiveness of past management strategies, such as the initiation of wastewater treatment. Since 1986, the National Oceanic and Atmospheric Administration's Mussel Watch Program and California Surface Water Ambient Monitoring Program have periodically collected data on chemical contaminants and biological indicators of water quality in the Tijuana River Estuary. This project builds on these past monitoring efforts and established methodologies to assess status and trends of contaminants in organisms and sediment. This work was accomplished by conducting a thorough review of available datasets and literature to document past changes in the estuary and refine sampling approaches. Sampling was conducted at three locations in the Tijuana River Estuary to assess spatial variability during both the wet and dry seasons. Overall, this information will improve our ability to document and interpret long-term trajectories of change of contaminants in the ambient environment and key taxa. This project was developed in coordination with key stakeholders, and the results will offer communication and management tools depicting the estuary's ecosystem health over time.

01.01.10 In Silico Computational Chemistry to Predict Accessible and Reactive Areas for Benzo[A]Pyrene Metabolites in Nucleosomes

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Benzo[a]pyrene (BaP) and its metabolite (+) benzo(a)pyrene-7,8-dihydrodiol 9,10-epoxide (BaP-DE) have been observed to induce transgenerational bone impairment in fish. It is hypothesized that both DNA methylation and histone tail modifications in the nucleosomes of ancestral germ cells are contributing factors to generate the observed transgenerational phenotype. The mechanism for histone tail modification induced by the toxic BaP-DE isomer is unknown. We present here a computational chemistry study on the direct impact of the presence of BaP-DE on the H3 histone tail in the chromatosome. Since histone tails are subject to post-translational modifications (PTMs) like de-/methylation and de-/acetylation which control the histone tail function in gene regulation and chromatin structure, homology modeling was used to construct different H3 histone tails containing multiple combinations of PTMs to replicate the configuration of the nucleosome at different states. A replica exchange molecular dynamics (RE-MD) approach was implemented to identify the most suitable conformation for each of those H3 histone tails, followed by a combined quantum-mechanics/molecular-mechanics (QM/MM) energy minimization. Each newly created nucleosome was then subjected to docking simulations using BaP-DE as ligand, identifying the most suitable recognition pocket regions by using a scoring function based on the AMBER force field. Finally, the BaP-DE /nucleosome systems were again exposed to energy minimization at the QM/MM level. The impact of BaP-DE docking on the H3 histone tail conformation and stability of the entire nucleosome was determined. In general, the H3 histone tail hydrophobicity was affected by the presence of methyl and acetyl groups, suggesting a dramatic change in its conformation after each modification. The intercalation of BaP-DE on the H3 tail functionality before and after those modifications, revealing its role in this critical process. These present in silico study allowed to identify BaP-DE binding priority sites and predict BaP-DE induced histone modifications putatively impacting the gene expression process. The results will inform functional studies in germ cells and osteoblasts in the biological Medaka fish model and enhance the understanding of epigenetic mechanisms involved in transgenerational inheritance.

01.01.11 Predicting the Toxicity of Emerging Pollutants: Interactions of Polyhalogenated Carbazoles With Human Androgen Receptor Via Molecular Docking

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Polyhalogenated carbazoles (PHCZs) are persistent chemical pollutants that are increasingly detected in different environmental matrices. They are structurally and chemically similar to other persistent organic pollutants (POPs), which are capable of disrupting the endocrine systems. However, PHCZs such as 3-Chloro-9H-carbazole (3-CCZ), 3-Bromo-9H-carbazole (3-BCZ), and 36-Dibromo-9H-carbazole (36-BCZ) are rarely discussed in the context of their adverse effects on human health. Using molecular docking to investigate the relative toxicity of these PHCZs with the human androgen receptor (AR), this study finds that 36-Dibromo-9H-carbazole and 3-Bromo-9H-carbazole are potential AR antagonists with the former being more toxic than the latter. This finding is on account of the presence of both *Asn705* and *Thr877* in the hydrophobic interaction of 36-BCZ, while only *Thr877* is found in the hydrophobic interaction of 3-BCZ. Hence, PHCZs with higher bromine substitutions are more likely to be endocrine disruptors. Moreover, the binding sites of all these PHCZs under study with the human androgen receptor are similar to that of the androgen (agonist). Therefore, this study suggests that PHCZs may readily penetrate and disrupt the human androgen receptor (AR), providing groundwork for future research studies and experimental validation on the molecular docking employed.

01.01.12 Application of Bioaccumulation Data in Ecological Assessments Under Canada's Chemicals Management Plan

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Bioaccumulation data have been used in the risk assessment of chemicals as part of the Chemicals Management Plan (CMP) under the *Canadian Environmental Protection Act, 1999* (CEPA) for over two decades. The science of bioaccumulation and its application in risk assessments has evolved during this time to be more versatile and comprehensive. The range of data for bioaccumulation consideration has been expanded from aquatic to terrestrial species, and with a broader application from simply evaluating bioaccumulation potential of a substance to informing ecotoxicity and exposure. This poster describes how bioaccumulation data are applied in risk assessments moving forward. One case study will illustrate the application of bioaccumulation data in risk assessment in the context of version 2 of the Ecological Risk Classification of organic substances (ERC2), a tool developed by Environment and Climate Change Canada (ECCC) for the purpose of prioritizing approximately 12 000 chemicals on the Domestic Substances List (DSL) that did not meet the categorization criteria used to prioritize substances for assessment in 2006 under the CMP. The other case study will highlight how bioaccumulation data, empirical or modelled with consideration of biotransformation potential, can be applied in an assessment, for example, to refine tissue residue estimation to allow for characterizing ecotoxicity potential of a substance, which can contribute to the derivation of a predicted no effect concentration. These applications use bioaccumulation data as part of the overall weight-of-evidence analysis, which enhances science-based decision-making on chemicals management.

01.01.13 A Review of International Methods to Derive Water Quality Guidelines for the Protection of Aquatic Life

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To establish new regional or national Water Quality Guidelines (WQGs) for the protection of aquatic life, a critical understanding of existing international WQGs and associated derivation methods becomes necessary for their adoptions or modifications. An international collaborative study critically reviewed the methods used to derive WQGs for the protection of aquatic life in five countries, including Australia, Belgium, Canada, the Netherlands, and the United States of America (USA). Each country adopted various combinations of methods based on species sensitivity distributions, assessment factors, geometric mean calculations, dietary exposure modelling, and other chemical-specific and general models. The countries also differed in their approaches to incorporate bioaccumulation (indirect ecotoxicity), background, and toxicity modifying factors (e.g. temperature, salinity, and pH) in their WQGs. Other factors that influence chosen derivation methods include data availability, chemical-specific mode of toxic action and regulatory or environmental priority. The study highlighted the role of policy in setting WQGs and the importance of understanding the science and data used to underpin the WQGs. The information generated from this study will allow an informed and transparent selection of international WQGs by those jurisdictions looking to establish their WQGs and will ensure scientifically robust and appropriate values are adopted for water quality management.The presentation will provide an overview of the global methods to derive WQGs for the protection of aquatic life and discuss considerations for their adoptions and applications by other countries. Appropriate examples will be provided to discuss the importance of understanding the science and data used to support the WQGs, which is critical and integral to managing water quality for the protection of aquatic life.

01.01.14 Per- and Polyfluoroalkyl Substances (PFAS) Community-Level Biomonitoring in Pennsylvania

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The Pennsylvania DOH conducted per- and polyfluoroalkyl substances (PFAS) biomonitoring in two affected communities to evaluate a CDC-developed PFAS biomonitoring toolkit. DOH collected serum samples, demographics, and exposure history from 235 randomly selected residents. DOH also collected samples of drinking water and household dust from 14 of the selected households (25 participants). DOH communicated individual results and community-level results to participants via letters and community meetings and sought feedback on results communications via a follow-up survey. DOH's objectives were to (1) assess the serum levels of selected PFAS, (2) compare the community PFAS levels with the national averages for 2013-2014, (3) analyze the relationship between serum PFAS levels and various demographic and exposure characteristics, (4) analyze the relationship between serum PFAS levels and those in household dust and drinking water and, (5) receive feedback on DOH's communication approaches. Of the 11 PFASs analyzed, only perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), perfluorohexanesulfonic acid (PFHxS), and perfluorononanoic acid (PFNA) were consistently detected in the serum samples. The average serum levels of PFOA, PFOS, PFHxS, and PFNA were 3.13, 10.24, 6.64, and 0.74 micrograms per liter (µg/L), respectively. Overall, 75, 81, 94, and 59 percent of the participants had levels exceeding the 2013-2014 national averages for PFOA, PFOS, PFHxS, and PFNA, respectively. Sex, age, employment in the study area, public water area, daily tap water consumption, and length of residence in the study area were found to be associated with serum PFAS levels. PFOA, PFOS, PFHxS, and PFNA levels in dust samples ranged from 3.94-522.00, 3.20-1,110.00, 1.44-862.00, and 1.24-276.00 ng/g, respectively. The correlation coefficients between dust and serum PFAS were 0.22, 0.26, 0.33, and 0.23 for PFOA, PFOS, PFHxS, and PFNA, respectively ($p \geq 0.05$ for all). Correlations between PFAS levels in water and serum PFAS levels were not analyzed as ≥50% of water test results were non-detects. Participants' suggestions for future biomonitoring results communication included providing a list of potential conditions associated with high PFAS exposure and the symptoms of early onset of disease, links to additional scientific studies, and a list of physicians schooled in environmental health. This work laid the foundation for additional health studies in this community.

01.01.15 Comparison of the Impact of Fine Particulate Matter (PM2.5) Filter Extraction Techniques Across Seasons on Oxidative Potential Analysis

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Fine particulate matter ($PM_{2.5}$) exists as a mixture of naturally and anthropogenically generated air pollutants that are 2.5 microns or smaller. $PM_{2.5}$ is hypothesized to induce oxidative stress, and has been linked to acute and chronic adverse health effects. $PM_{2.5}$ is collected onto filters by state environmental agencies as part of EPA-mandated air quality monitoring. Prior to toxicology 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 oxidative potential of the samples. Prior to extraction, black carbon data was collected for each filter. The filter extraction techniques used were sonication in: 1) methanol 2) DCM, 3) DI water or 4) 0.9% saline. Extraction was performed on co-located $PM_{2.5}$ filters collected by the Arkansas Department of Environmental

Quality at four locations during the same 24-hour collection period, across seasons. Filter quadrants were extracted with the appropriate solvent, 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 the winter (January 4th and 22nd, 2012) identified significant differences in DTT consumption (nmol/min/m^3) occur between extraction methods measured in the oxidative potential assay. Chemical analysis of extracts will also be performed using ICP-MS to characterize elements present and for statistical comparisons to the DTT assay results. We anticipate that these results from previously collected yet untested filters will identify the importance of filter extraction in interpretation of oxidative potential results and studies designed to improve understanding of the health effects following $PM_{2.5}$ exposure. This work will ultimately support the utilization of previously collected $PM_{2.5}$ samples to develop targeted regulatory standards based on non-concentration based measurements.

01.01.16 Field Testing of Prototypes of Actively Shaken In-Situ Passive Sampler Platform for Passive Sampling

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Passive sampling for sediment porewater has emerged as a very promising approach, but in situ measurements are complicated by slow mass transfer of strongly hydrophobic compounds. The primary resistance to mass transfer arises in the sediment side where a static boundary layer develops close to the polymeric passive sampling material. The slow mass transfer results in under-equilibrated passive sampler measurements that need to be corrected for equilibrium, typically by extrapolation of the loss kinetics of performance reference compounds. Such corrections are prone to large errors, especially when deviation from equilibrium is large. Our current research aims at addressing the challenge of slow mass transfer by disrupting the external aqueous boundary layer around an in-situ passive sampler using periodic mechanical vibration. A total of three new prototypes of vibrating passive samplers have been developed to address the objectives of this project. Key motivations were to increase the size of the sampling device, as compared to the first prototype built for proof-of-concept, increasing the size of the motor, making them more robust for deployment in the field, and keeping them low-cost. The first prototype (P1_VibraSP3™) was developed in collaboration with Geosyntec. This prototype is still too small to deploy 1 g of PE per sampler. Two additional vibrating passive samplers (prototypes P2_12V110G and P3_24V125G) were developed at UMBC that are larger in size and power to allow the deployment of 1-3 g of PE passive samplers. Additionally, P2 and P3 could accommodate a 3-axis vibration datalogger on one of the fins to keep track of continued vibration. The prototypes were tested by deploying in laboratory set-up sediment mesocosm up to 14 days and also in the field at Anacostia River. P1_VibraSP3™ platform was intact and fully-functioning indicating a successful performance of the device. Vibration data from the datalogger demonstrated that the prototypes P2 and P3 vibrated up to 14 days of deployment and the average amplitude of vibration of the leakproof devices within the sediment ranged between 2-5G over the entire period. Overall, the results of the deployment studies confirmed that the vibrating passive samplers enhanced the kinetics of uptake of the PCB congeners.

01.01.18 Updating Research Concerning Continuing Ecological Effects of Deepwater Ocean Dumping of DDT Wastes Into the Southern California Bight

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The nation's largest DDT manufacturer, Montrose Chemical, adjoining coastal Los Angeles, California, manufactured thousands of tons of the insecticide DDT from 1947 to 1982. Much of the DDT "caustic" waste from the DDT plant until the 1960s was transported to the Los Angeles County Sanitation Districts (LACSD) wastewater treatment plant and ocean outfalls near the Palos Verdes (PV) Shelf. During the 1990s, EPA. NOAA, and US DOJ retroactively launched a Natural Resource Damage Assessment (NRDA), Superfund listing, and subsequent "DDT trial" which took place in 2000 in Los Angeles. This trial focused exclusively on DDT residues in sediments located within a 17 square mile Superfund "site", much of it in shallow subtidal waters. This designation by EPA in 1996 was based on wastewater discharges from LACSD. The trial outcome was that Montrose Chemical negotiated a large monetary settlement of approximately $150M earmarked for restoration of Santa Monica Bay, including limited cleanup, educational programs focused on DDT exposures, and related activities. However, none of these legal or regulatory actions were specifically related or intended to follow up the widespread, unfortunate practice of deepwater ocean dumping of DDT wastes from the Montrose plant into deepwater dumpsites located in and around Santa Monica and San Pedro Basins to depths of approximately 1,000 meters. An important comparison is that the estimated total mass of DDT of up to 1,535 tons of DDT from the deepwater dumping was *approximately equivalent* to the estimate of up to 1,450 tons released through the wastewater outfalls along the PV Shelf. Sediment measurement of total DDT in sediments from both locations were also *approximately equivalent*, further substantiating the importance of this separate waste stream. Our work distinguishes the two distinct DDT waste streams (i.e. wastewater discharges and deepwater ocean dumping) and focuses on what is known of the potential ecological effects to marine mammals, fish, zooplankton, and other marine biota from deepwater ocean dumping of DDT waste throughout the Southern California Bight.

01.01.19 Evaluation and Standardization of a Whole Effluent Short Term Growth Toxicity Test Method Using Daphnia magna

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In 2009, USEPA's Office of Research and Development (ORD) published a paper titled, Development and Validation of a *Daphnia magna* Four-Day Survival and Growth Test Method. The test results were compared to performance criteria and results from 7-d survival and reproduction tests with *Ceriodaphnia dubia*, to assess the level of comparability of the two methods. Results from tests using the 4-d *D. magna* survival and growth method showed that this method produced consistent results with various reference toxicant materials and provided data that were both reproducible and useful for detecting potential toxicity in aquatic environments. Since that publication, there has been an increased interest in using the *D. magna* method in Whole Effluent Toxicity (WET) tests. In 2020, ORD began re-evaluating the method in a bi-laboratory approach at USEPA laboratories in Cincinnati, OH and Duluth, MN as well as a collaboration with National Council for Air and Stream Improvement (NCASI). As part of the first phase of testing, ORD is currently evaluating various test conditions such as test duration (4-d vs 5-d), number of treatment replicates (n=4, 5, or 6 replicates/treatment) in a five concentration test, test food types and levels, and the general culture conditions. Various test and culture conditions may contribute to test variability and the ability to meet the original performance criteria. In our studies, we are using the performance criteria of 80% control survival with a mean final dry weight of ≥10X greater than mean initial test organism dry weight. Preliminary results indicate a 4-d procedure is reproducible in within-lab testing with KCl, with test evaluation at other laboratories currently underway. Demonstration of an acceptable level of intra- and inter-laboratory variability is required for the acceptability and approval of a WET test method. A second phase will involve using the test conditions determined to be reproducible in Phase 1 to examine *D. magna* survival and growth response to a larger suite of reference toxicants (KCl, $ZnSO_4$, NH_4Cl, phenol and bifenthrin) and the consistency of responses over multiple tests (n=3 tests/toxicant) and at least five effluents. This presentation will highlight the latest progress of Phase 1 outcomes.

01.01.23 Using Time Course of Histopathological and Behavioural Changes to Identify Target Organ Toxicity in a Rat Model Following Oral Exposure to Contaminated Groundwater

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Groundwater containing a complex mixture of known and unknown contaminants was collected from an industrial site for the characterization of toxic effects. A two-tiered approach using a broad screen of potential target organs was undertaken, with the first tier using a time course of histopathological, physiological and behavioural responses after 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). A transient, significant increase in white blood cell count was seen in the day-7 experimental group compared to the control. Blood smears showed occasional lymphocytosis and neutrophilia, but these did not differ significantly with treatment at any time-point. Moreover, 40% of the experimental rats at all time points had bite cells. Bone marrow smears showed hypercellularity after 56 days of contaminated drinking water consumption compared to control. While gross behaviour appeared normal, a novel object exploratory test showed an increased duration and number of interactions in the 56 day-exposed rats. In summary, this study provides a framework for future studies of unknown, complex mixtures to characterize a broad range of toxic effects and target organs. The evidence of immunotoxicity, hematotoxicity, and neurological toxic effects, will be explored in more mechanistic detail with a targeted dose-response experiment in the second tier of experiments.

01.02 Advancing Use of Behavioral Studies in Regulatory Assessment of Contaminants and the Environment

01.02.01 Advancing the Use of Behavioral Endpoints to Link Chemical Exposure to Biological Phenotypes and Enabling Dissemination Through Data Science and Visualization

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The OSU/PNNL Superfund Research Program represents a longstanding collaboration to quantify Polycyclic Aromatic Hydrocarbons at Superfund sites in the Pacific Northwest and assess their potential impact on human health. To link chemical exposure to potential impacts on biological systems, we have leveraged the zebrafish embryonic development model to provide quantitative measurements of phenotypic changes from toxicants. Toward this end, we have identified several hundred chemicals found at Superfund sites and measured the effect of these same chemicals in zebrafish, creating a rich dataset that links environmental exposure to phenotype. High-content imaging of larval zebrafish behavior have enabled the addition of several functional assessments of chemical toxicity that go beyond traditional morphological endpoints. However, in order for these dynamic activity measurements to be useful for dose-response modeling, they need to be transformed into adverse events that quantify the impact of chemical exposure over time compared to normal fish behavior and at single embryo resolution. We have developed algorithms that transform zebrafish behavioral measures that capture embryonic photomotor response, larval photomotor response and repeated measures of larval photomotor response. We have identified several distinct phenotypes as endpoints to quantify the behavioral impact of chemicals on zebrafish development. We have also implemented dose-response modeling pipelines to calculate benchmark dose parameters for these data that enable the comparison of potency across chemicals and phenotypes. Finally, we describe a publicly accessible data portal that provides public access to these datasets, including linkage between environmental concentrations and laboratory-tested concentrations of chemicals, using an interactive web site designed to enable exploration and re-use of this data by the scientific community at http://srp.pnnl.gov.

01.02.02 How Multiple Behaviors of United States Environmental Protection Agency (USEPA) Ecotoxicity Test Organisms Affect Suitability for Use in a Sediment Avoidance Test

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Soil avoidance tests have been developed for earthworms, Collembola, and other soil organisms, based on the fact that these organisms are sensitive to many toxic contaminants and have the ability to avoid areas of contamination. Versions of an earthworm soil avoidance test have been increasingly used in risk assessment since its development in 1996. Avoidance testing with sediment organisms have less of a history of development and use than earthworm soil avoidance, but this behavioral endpoint has similar potential as an indicator of population level effects, for use in decision-making, and for incorporation into regulatory assessments of chemical contaminants. We assessed the potential for use of the freshwater aquatic macroinvertebrates (*Chironomus dilutus* larvae, *Hyallela azteca*, and *Lumbriculus variegatus*) used in standard U.S. EPA sediment toxicity and bioaccumulation methods (EPA Methods 100.1, 100.2, and 100.3), for use in sediment avoidance testing. Previous testing with sediments from contaminated sites has provided evidence that avoidance tests have the potential for increased sensitivity to contaminated sediments relative to standard toxicity tests for some of these test organisms. A current focus of testing is how behaviors other than avoidance of contaminants - feeding habits, predator avoidance, mating, phototactic responses to light, but especially mobility - affect avoidance testing. These additional behaviors should be taken into account in developing test procedures, interpreting results, and most critically in choosing suitable test organisms. Mobility tests with reference sediment, with a null hypothesis that the organisms will spread themselves relatively evenly in chambers, showed differences in mobility between the organisms. In 24-hour tests *Lumbriculus* (p=0.236) and *Hyallela* (p=0.327) both showed good mobility, whereas *Chironomus* (p=0.002) did not. We also discuss development of test procedures that can help prevent other behaviors (e.g. phototaxis, forming of colonies (*Lumbriculus*), pairing (*Hyallela*)) from significantly affecting test results. Avoidance of contaminated sediments by aquatic macroinvertebrates has the potential to provide a fast, sensitive, low-cost means of assessing risk from contamination and for evaluating the success of sediment remediation efforts at a range of contaminated sites (Superfund, Great Lakes Areas of Concern (AOC), mining).

01.02.03 Utility of Larval Zebrafish Behavior: Comparison of Behavioral and Developmental Toxicity

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The U.S. Environmental Protection Agency has developed an early life stage vertebrate assay which assesses both developmental endpoints and behavioral endpoints in larval zebrafish (*Danio rerio*) exposed to chemicals during development. This larval toxicity assay was tested to screen 88 chemicals to determine the potential chemical influence on 6 day old zebrafish development or behavior at concentrations ≤ 120 µM. These chemical screens resulted in varying relationships between developmental (e.g. mortality or morphology changes) and behavioral toxicity. Some of these relationships included behavioral alterations while no developmental toxicity was observed at any concentration or observing behavioral changes magnitudes below chemical concentrations that caused developmental toxicity. For example, some chemicals (e.g., paraquat, cyclophosphamide, or 6-aminonicotinamide) elicited behavioral effects without causing any developmental toxicity (highest concentration was 120 µM). In addition, amphetamine induced significant hyperactivity at low concentrations 0.4-1.2 µM in both light and dark photoperiods while developmental effects were only observed at 120 µM. Finding behavioral changes either in the absence of developmental effects or at concentrations far below those eliciting developmental changes highlights the sensitivity of behavior as a toxicological endpoint. Many, but not all, of the chemicals causing these responses are known cholinesterase inhibitors or other types of neuroactive compounds. Behavioral changes can affect an organism's ability to feed and reproduce, or increase the susceptibility to predation--all of which may influence the overall health and success of a population. Therefore, incorporating behavioral changes as an indicator of chemical exposures into traditional environmental studies should strengthen the robustness and sensitivity of assessments. *This abstract may not necessarily reflect official Agency policy.*

01.02.04 Development of a Reproducible Behavior Assay to Screen for Chemical Effects

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In ecotoxicology, behavioral endpoints are rarely used in regulatory framework when evaluating the effects of chemicals on organisms in aquatic ecosystems. Typically, traditional endpoints—reproduction, growth, and survival—are used for regulatory evaluation of organismal response to chemical exposure. Traditional endpoints are often used for regulatory risk assessment because they are relatively simple to measure and can provide a direct link to population-level responses. However, prior research has shown toxicity of chemicals can produce complex effects on organisms that are difficult to measure using traditional endpoints. Thus, behavior may be a more robust and sensitive biological endpoint to quantify effects of chemical exposure for regulatory risk assessment because behavioral responses to chemical exposure can be detrimental to an individual's fitness and ultimately, the survival of a species. However, prior research has indicated that reproducibility of standard methods in behavioral assays for use in regulatory risk assessment are poorly characterized. The goal of our research was to develop a reproducible behavioral assay for standard screening of chemical effects for regulatory risk assessment. Behavioral assays were performed using embryonic and larval fathead minnow (*Pimephales promelas*) as model organisms because 1) species life-history is well characterized, and 2) because they are widely distributed across watersheds in North America. Using a Viewpoint Zebrabox and software, fish movement served as proxy for behavior and was quantitatively used to assess sublethal effects caused by chemicals. Specifically, we used distance moved in response to changes in photoperiod (light vs. dark) across varying concentrations of three chemicals—phthalates, metals, and selective serotonin reuptake inhibitors (SSRIs). Our results increase our theoretical and applied knowledge of how behavior can be used a tool for standard assessment for regulatory risk assessment of chemicals.

01.02.05 Bioaccumulation and Behavioral Impacts of Low Dose, Food-Borne Methyl Mercury Exposure in Larval Zebrafish

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Methylmercury is a persistent, highly toxic environmental contaminant that is widespread in aquatic environments. It is highly bioaccumulative in fish tissues and can cross the blood-brain barrier, resulting in numerous health consequences including behavioral, neurological, and immunotoxic effects. Prior studies assessing the effects of MeHg toxicity in zebrafish (*Danio rerio*) have focused on waterborne routes of exposure, and little is known about the impacts of MeHg ingested through feed at environmentally relevant exposures. In this pilot study, we are investigating the potential bioaccumulation and behavioral impacts of low dose, food-borne routes of MeHg ingestion in zebrafish larvae. Larval zebrafish will be fed a standard zooplankton (*Brachionus plicatilis*) diet starting at 5 dpf until 35 dpf, which corresponds to the onset of feeding and completion of sexual differentiation, respectively. Larvae from treatment groups will instead be fed zooplankton incorporated with environmentally relevant concentrations of MeHg (1, 10, and 100 ppb) and will be assessed for potential growth and behavioral effects (e.g., startle response, swim speed) during the 30 day time period. At the conclusion (35 dpf), the remaining larvae will be collected to determine levels of MeHg bioaccumulation for the three experimental groups. We hypothesize that this natural route of MeHg exposure will lead to differential bioaccumulation and altered behavioral responses during critical early life stages of zebrafish. This study will address potential cognitive impairments and additional fish health parameters that are likely to effect fitness in wild fish.

01.02.06 Photosensitivity and Predator Avoidance in Pyrethroid-Resistant Hyalella azteca

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Hyalella azteca is a species of freshwater invertebrate and a vital component to many aquatic ecosystems, serving as a primary food source and indicator species that highlights environmental issues. One such ecological problem, and the focus of our research, is pesticide exposure. Increased pesticide use in agricultural and urban landscapes has caused certain *H. azteca* populations to develop resistance to pyrethroid pesticides, which may have adverse effects on *H. azteca*'s chemosensory and photic reflexes. In addition, a genetic bottleneck can be created due to these elevated exposures that decreases the population's diversity and stability. Our research explores how resistance mutations in *H. azteca* alter their responses to certain stimuli by comparing how resistant and non-resistant populations react to light, chemical predator cues, physical/chemical hybrid stimuli, and a live predator chemical cue. Tests were conducted in dishes containing 1000 mL of water and 10 *H. azteca* in each trial. A total of 10 trials per physical stimuli were conducted. A lightbox was used for the physical stimuli, while L-histidine, L-serine and live predator chemical cues were used as chemical stimuli. Finally, to determine how the responses to stimuli are affected by pyrethroid exposure, *H. azteca* were exposed to permethrin, a neurotoxin, at a non-lethal concentration. Each test was paired with a respective control trial to account for random behaviors unassociated to the experimental element. Our preliminary data suggests that non-resistant populations have much stronger and faster reactions compared to the resistant populations. We have especially seen these results in our light stimuli trials. As we continue to collect data and draw our final conclusions, we are focused on how the changes in behaviors caused by resistance mutations can affect *H. azteca* populations and their ability to respond to threatening stimuli.

01.02.08 Identifying Drinking Water Metal Contaminant Mixture Risk by Coupling Zebrafish Behavioral Analysis With Citizen Science

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Contaminated drinking water is an important public health consideration in New England where well water is often found to contain arsenic and other metals such as cadmium, lead, and uranium. Chronic or high level exposure to these metals have been associated with multiple acute and chronic diseases, including cancers and impaired neurological development. While individual metal levels are often regulated, adverse health effects of metal mixtures, especially at concentrations concentrations considered safe for human consumption remain unclear. Here, we utilize a multivariate analysis that evaluates the chemical constituents of 92 drinking well water samples, collected in Maine and New Hampshire, with behavioral outcomes using the zebrafish model. To collect these samples, a citizen science approach was used, that engaged local teachers, students, and scientific partners. Our analysis of 4,016 mixture combinations provided evidence that changes in zebrafish behavior are highly mixture dependent and indicate that certain combinations of metals are more significant drivers of behavioral toxicity. As an example, lead was present in 67% of mixture combinations associated with a decrease in activity, while uranium and cadmium were more prevalent in mixtures associated with an increase in activity. Arsenic was equally present in mixture combinations associated with both behavioral phenotypes and most often at concentrations below the maximum allowable limit of 10 parts per billion. Our data emphasize the need to consider low-level mixture effects and provide a framework for a more in depth analysis of drinking water samples as well as those found in the environment, given that aquatic organisms are likely exposed to similar metal mixtures. We also provide evidence for the efficacy of utilizing citizen science in research, as the broader impact of this work is the expansion of knowledge among local communities of improving their own water quality.

01.02.09 Effect of Microplastics on Growth and Reproduction of Eisenia fetida

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Plastic is a versatile, hygienic, light weight and highly durable product which has brought social benefits, technological and medical advances. The plastic present in the environment loses its integrity through thermo and photo-oxidative processes leading to its fragmentation into smaller pieces. Microplastics (MPs) are synthetic polymer matrix, with different shapes and sizes, ranging from 1µm to 5µm, ubiquitous in nature causing severe environmental pollution. Sewage sludge, agricultural mulching and plastic waste are primary sources for the accumulation of MPs in soil. Earthworms, the essential organisms in eco-toxicological studies, are the soil mega fauna which have an important role in breakdown of organic waste and formation of soil, as a result of which MPs too move to the deeper horizons of soil. The current study evaluated the effect of MPs on growth and reproduction of *Eisenia fetida,* by exposing the earthworms to six doses of microplastics -T1 (1g/kg), T2 (5g/kg), T3 (10 g/kg), T4 (15 g/kg), T5 (20g/kg) dry wt for 28 days. The earthworms were reared in artificial soil as per OECD guidelines to check effect of MPs on body weight, cocoon numbers laid, change in hatchling number and change in the activity of glutathione-S-transferase as indicators of oxidative stress related toxicological effects. The results indicated an inversely proportional effect on body weight -the weight decreased as the dose of the MPs increased. However no significant change in mortality, cocoon production and hatchlings was recorded in any of the doses of MPs tested. Variation was observed in GST activity in *E. fetida* at concentrations T1, T2 and T3 of MPs with an initial increase in GST activity followed by its decrease with the increase in duration of exposure to MPs indicating a reactive oxidative stress to the contaminant, but the steadying off of the GST activity is an indication of no toxic reaction on the earthworm in all doses of treatment. It is concluded that MPs have no effect on the reproduction of the earthworm at the selected concentrations, though at the initial days of exposure some toxic reaction is recorded by the earthworm *E. fetida*. At higher level T5 MPs may result in adverse effects on earthworm physiology which may be indicative of similar reaction among the soil fauna. This study provides a lead that the MPs may not have a detrimental role on soil fauna. However, more detailed findings can be employed to study the effect of MPs on soil fauna.

01.03 Adverse Outcome Pathways – Developing a Community of Practice

01.03.01 AOP Reports: A New Way of Publishing AOPs in Peer-Reviewed Scientific Journals

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In 2012, the Organisation for Economic Co-operation and Development (OECD) launched the Adverse Outcome Pathway (AOP) Development Programme. The objective of this programme is to support and promote the development of AOPs by providing guidance and coaching for AOP developers, an OECD recognized technical review and endorsement process, and publication of AOPs in a dedicated OECD publication Series. Ultimately, the aim is to increase confidence in the quality and scientific credibility of AOPs that is required for the use of AOPs by regulators and risk assessors. The AOP Development Programme has grown significantly over the past decade. One of the challenges associated with that success is an increasing pressure on resources for managing and coordinating the rigorous scientific review process. At the same time, professional recognition for the significant scholarly investment required for formal AOP development and subsequent technical review has often been viewed as insufficient to warrant the effort of proceeding through technical review and endorsement, particularly for academics whose productivity is typically measured via publications in peer-reviewed journals. To mitigate these challenges, an initiative was taken to develop a strategy for cooperation between the OECD and scientific journals. In this model, journal organized scientific review of AOPs documented in the AOP-Wiki, accompanied by a narrative "AOP Report" journal article, can substitute for the OECD scientific review as long as reviews are conducted in a manner consistent with the principles outlined in the OECD guidance on the scientific review of AOPs. Amongst others, this implies an open review process in which the identity and comments of the reviewers are made publicly available. Upon acceptance, such AOPs can immediately be considered for OECD endorsement. Overall, this novel AOP publication strategy provides AOP authors with the career recognition associated with a peer-reviewed publication while supporting the OECD by engaging the journals' editorial infrastructure to manage the scientific reviews. Two journals, Environmental Toxicology & Chemistry and Environmental and Molecular Mutagenesis, are currently pioneering this approach with the first AOP Reports being published in 2021. This AOP publication model is open and available to the entire scientific community involved in AOP development. The contents of this abstract neither constitute nor necessarily reflect USEPA policy.

01.03.02 Development of Quantitative Adverse Outcome Pathways to Facilitate Next Generation Risk Assessment of Mitochondrial Toxicants

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The Adverse outcome pathway (AOP) framework has been introduced to better organize (eco)toxicological information to assist risk assessment and regulatory decision-making. A quantitative AOP (qAOP) is an evolved form of AOPs that captures the quantitative key event relationships (dose-response and response-response relationships) and biological complexities (temporal variations, species sensitivities) in a mathematical model, allowing predictions of regulatory-relevant adverse effects based on key events measured at lower levels of biological organization (molecular and cellular) using cost-efficient new approach methodologies (NAMs). A mature qAOP model in combination with environmental exposure information can be readily used for hazard and risk predictions and greatly reduce the needs for *in vivo* (eco)toxicity testing. By introducing our recently launched RiskAOP project, this presentation aims to share some experiences and strategies on qAOP development and application. RiskAOP is a proof-of-concept project to develop qAOPs and evaluate their applicability in risk assessment, using chemical-mediated mitochondrial dysfunction in commonly used regulatory testing species (plant, crustacean and fish) as a demonstrative case. The main goals of the project are to: develop new AOP networks and associated NAMs for efficient testing of mitochondrial toxicants; generate new experimental data for mechanistic and quantitative understanding of chemical-mediated mitochondrial dysfunction across different trophic levels; develop novel qAOP network models and evaluate their robustness and applicability for hazard assessment towards regulatory needs; propose new strategies for qAOP assisted risk assessment and regulatory decision-making. A network of AOPs linking uncoupling of mitochondrial oxidative phosphorylation and growth inhibition has been developed, with one AOP currently under review by the OECD (AOPWiki, AOP#263). New experimental data will be generated for qAOP construction using Bayesian networks and structural equation modeling. Risks of chemicals will be predicted based on a combination of early key events in an AOP and exposure information via the qAOP models. Ultimately, recommendations on how qAOPs and NAMs can facilitate screening, classification, prioritization, and risk assessment of chemicals will be provided. This project is funded by the Research Council of Norway (grant 301397) and supported by the NIVA Computational Toxicology Program (NCTP).

01.03.03 A Modular Approach to Adverse Outcome Pathway Development: Peroxisome Proliferator-Activated Receptor Alpha Agonism Impairs Fish Fertility

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As per- and polyfluoroalkyl substances (PFAS) have been increasingly found in surface waters and sediment, there has been growing demand to better understand the potential ecological effects of these chemicals. Recent high-throughput screening of over 140 PFAS has shown peroxisome proliferator-activated receptor alpha (PPARα), a nuclear receptor that regulates transcription of various genes involved in fatty acid beta oxidation and lipid homeostasis, is one of the prominent biological targets affected by multiple PFAS. To better understand the ecological significance of PPARα activation, we reviewed the scientific literature with the aim of developing one or more adverse outcome pathways (AOPs) relevant to PPARα agonism. In this presentation, we will focus on PPARα agonism and its potential effect on fertility. Effects of observed in multiple studies involving exposure to fibrates, synthetic ligands of PPARα prescribed to reduce cholesterol in humans, provided weight of evidence (WOE) for connecting PPARα agonism to decreased cholesterol (Key event relationship[KER] 2073) and decreased cholesterol to decreased 11-ketotestosterone (11-KT; KER 2072). The current presentation details the development of the WOE for the last two additional KERs (2076 and 2274), which connect decreased 11-KT to impaired spermatogenesis and impaired spermatogenesis to impaired fertility utilizing a KER by KER approach. This approach began with a literature search utilizing AbstractSifter which pulls literature from Pubmed based on key search terms. Afterwards, literature was filtered based on relevance and compiled, maintaining both supporting and conflicting evidence to ensure objectivity. The WOE was then organized into concordance tables, evaluated for accuracy and completeness, and uploaded to the AOP wiki. Overall, this approach was effective in establishing plausibility and empirical support involving dose-response and temporal concordance while maintaining objectivity. While AbstractSifter was effective in pulling empirical support in support for the KERs, KER 2274 was primarily supported by studies that did not involve use of chemical stressors. This presentation summarizes the evidence assembled and some of the advantages and challenges encountered when implementing the approach. Overall, the KER by KER approach taken here was effective for collaborative AOP development. The contents of this abstract neither constitute, nor necessarily reflect, official USEPA policy.

01.03.04 Development of Adverse Outcome Pathway for PPARγ Antagonism Leading to Pulmonary Fibrosis Using Bradford-Hill Consideration and CTD Database

J. Jeong, J. Choi, University of Seoul / Environmental Engineering

Pulmonary fibrosis is regulated by transforming growth factor β (TGF-β) and peroxisome proliferator-activated receptor-gamma (PPARγ). Previously, an adverse outcome pathway (AOP) on PPARγ inactivation leading to lung fibrosis was developed. This study assessed the confidence of the overall AOP using the Bradford-Hill considerations based on the recommendation in the OECD Users' Handbook. The essentiality of the key events (KEs) and the biological plausibility of the key event relationships (KERs) were rated high overall. In contrast, the empirical support of the KERs was rated moderate. For a more extensive analysis of KERs, the relationships between gene, phenotype, and disease were analyzed through the Comparative Toxicogenomics Database. To validate this AOP experimentally, PPARγ (MIE) and TGF-β (KE1) inhibitors were used to examine the effects of downstream events after inhibiting their upstream event in both human bronchial epithelial cells (BEAS-2B) and primary cells (NHBE). Using the inhibitor and agonist of PPARγ, PPARγ inhibition (MIE) led to TGF-β activation (KE1), an increase in vimentine (KE3), and an increase in fibronectin (KE4). Similarly, with the inhibitor and agonist of TGF-β, TGF-β activation (KE1) led to an increase in vimentine (KE3) and an increase in fibronectin (KE4). The AOP on pulmonary fibrosis developed in this study will be the basis for the screening of inhaled toxic substances in the environment based on the existing knowledge of toxic mechanisms. Acknowledgement: This work was supported by a grant from the Korean Ministry of Environment through 'Environmental Health R&D Program' (2021003310005).

01.03.05 Evaluation of an Adverse Outcome Pathway Network for Thyroid Hormone System Disruption Across Taxonomic Groups

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Thyroid hormone system disrupting chemicals (THSDCs) are widely regarded as potential threats to human and environmental health. Thus, significant efforts have been made within both the human health and ecotoxicology communities to develop screening assays capable of identifying THSDCs and to describe adverse outcome pathways (AOPs) that link thyroid hormone system disruption (THSD) to adverse outcomes. In recent years, we developed a small, fish-specific, AOP network (AOPN) consisting of 5 AOPs linking the inhibition of enzymes important for the synthesis and activation of thyroid hormones to impaired swim bladder inflation. As an example of how the AOPN can be used to address the current gaps in methods for THSDC screening and testing in fish, a suite of assays along the AOPN that can be implemented in a tiered screening and testing approach was identified. When expanding the network to all AOPs for THSD in fish, mammals and amphibians (either endorsed or under development), a cross-species AOPN for THSD emerges. This broader AOPN provides a scientifically plausible and evidence-based foundation for the measurement of endpoints using fish and amphibian assays, as well as in vitro or in chemico assays, to predict outcomes in humans. In the present work, the taxonomic applicability of the AOPN was evaluated. By filtering the AOPN based on the currently described taxonomic domain of applicability of the AOPs (i.e., fish, amphibians or mammals), it was found that most AOPs have been developed with a focus on one specific taxonomic group and information on the taxonomic domain of applicability is often missing. We evaluated which AOPs have already been defined for more than one taxon, which AOPs are taxon-specific and thus irrelevant to other taxa (e.g., swim bladder inflation), and which novel pathways linking molecular initiating events to adverse outcomes (i.e., emergent AOPs) in a particular taxon are potential targets for dedicated AOP development. Based on this evaluation, we identified data gaps and prioritized AOP development efforts. Since developmental neurotoxicity (DNT) is often considered the most important outcome of THSD in humans, and no AOPs for DNT in fish and amphibians exist, DNT was highlighted as a priority for cross-species AOP development and a potential route to expedite the use of fish and amphibian assays for predicting effects in humans. *The contents of this abstract neither constitute, nor necessarily reflect, USEPA policy.*

01.03.06 A Machine-Readable Data Model for AOP Evidence and a Call for Community Feedback

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The committee that oversees the AOP Knowledgebase (AOP-KB) is considering modifications to the underlying database model that may improve the ease and transparency of how information are collected, organized, and retrieved, particularly with respect to automatable processes. Here, we present and invite community feedback on a pilot study that tests one of these modifications: a machine-readable model of the AOP evidence structure. For this pilot study, we designed a generalized data model based on the modified Bradford Hill criteria of causality for key event relationships (KERs). The model description included biological plausibility and various lines of empirical evidence such as dose-, temporal-, and incidence-concordance. A central theme was to ensure that evidence was transparently reported using easily queried structures. Accordingly, each unit of evidence could be explicitly linked to specific descriptions of stressors, measurement methods, biological domains, and publication references. We also developed a graphical user interface that streamlined manual input while enforcing fixed vocabularies. The model was tested by reconstructing the KER evidence of two OECD-endorsed AOPs (AOP #25: Aromatase inhibition leading to reproductive dysfunction; and AOP #131: Aryl hydrocarbon receptor activation leading to uroporphyria). Our objectives were to 1) evaluate the ease with which users could input information into the data model; 2) determine if coherent KER evidence structures could be represented; 3) test the transparency and accessibility of the resulting database using queries that cannot be conducted in the current AOP-KB; and 4) identify aspects of evidence collection that could potentially be automated. The SETAC community is invited to provide feedback on our proposed data model. Key questions for AOP developers include: Does this model make it easier to input AOP evidence? Does this model make you more likely to contribute? Key questions for regulatory end users include: What queries would you like to conduct in the AOP-KB? Does this model capture the information of greatest interest to you? We invite responses to these and other key questions, and contributions to the data model development discussion on the AOPwiki forum: https://aopwiki.org/forums/index.php. *The contents of this abstract neither constitute nor necessarily reflect US EPA policy.*

01.03.07 Quantitative Adverse Outcome Pathway Modeling for Prediction of Effects of Aqueous Per- and Polyfluoroalkyl Substance Exposure on Developing Zebrafish (Danio rerio)

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Per- and polyfluoroalkyl substances (PFAS) are environmentally stable and pose risks to human and ecological health through exposure to contaminated water. Their relatively high lipophilicity and hydrophobicity, in addition to a general resistance to biotransformation and excretion processes, favor bioaccumulation and may lead to toxic adverse outcomes (AOs), even at low exposure levels. The risks of PFAS exposure are hard to assess, as there are seemingly many disparate datasets to interpret and tie to AOs of interest. Adverse outcome pathways (AOPs) provide a conceptual framework to address this issue by linking a molecular initiating event (MIE) with an AO at the organism level through intermediate key events (KEs). One advantage of the AOP framework is that it is chemically agnostic, which motivates the following hypothesis: if an AOP can be populated with data, then organism AOs can be predicted entirely from MIE activity. Here we report a test of this hypothesis using a quantitative AOP (qAOP) modeling approach and discuss how the resulting model could inform PFAS risk assessment. The model describes an AOP

populated with data that connects Type II iodothyronine deiodinase (DIO2) inhibition (the MIE) to reduced Triiodothyronine hormone (T3) levels (the KE), and, finally, to an uninflated swim bladder (USB) in a population (the AO) of zebrafish (*Danio rerio*). The zebrafish are exposed to Perfluorooctanoic acid (PFOA), which is a strong DIO2 inhibitor, over a period of 120 hours post-fertilization. One challenge is that each link of the AOP is supported with PFOA exposure-response data, and we use Gaussian distributions to capture this conditional PFOA dependence and the overall statistical nature of the data. To recover the MIE to AO relationship, we use methods from Bayesian statistics to eliminate PFOA dependence from the model. As a result, we can make statistical predications for USB outcomes in zebrafish that depend only upon DIO2 inhibition. Although our model is developed from PFOA exposure data, we test its performance with Perfluorobutanesulfonic acid (PFBS), which moderately inhibits DIO2. As our model only requires DIO2 inhibition data for input, it can potentially inform screening assessments of numerous PFAS compounds. For assessment of PFAS that do not inhibit DIO2, our qAOP approach is general enough that it can be readily applied to other AOPs and may therefore appeal to the broader PFAS risk assessment community.

01.03.08 Application of Adverse Outcome Pathways for Identification of Multi-Walled Carbon Nanotubes Using Bayesian Network Model

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An adverse outcome pathway (AOP) is a framework that organizes the mechanistic or predictive relationships between molecular initiating events (MIEs), key events (KEs), and adverse outcomes (AOs). A Bayesian Network (BN) is a model that presents probabilities of the dependencies between any two variables in a graphical form. It is capable of diagnostic, predictive, and inter-causal reasoning. To develop the AOP of fibrosis and apoptosis, we conducted in vitro test on the biomarkers related to each KE using human liver cell (HepG2) and bronchial epithelium cell (Beas-2B). Cells were exposed to multi-walled carbon nanotubes (MWCNTs) at EC20 for 24 hrs. And qRT-PCR was conducted to generate the gene expressions data. With these data, BN model was learned using SAIAM, for identifying probabilistic causal relationships between the KEs. As a result, size is the main influencing factor on the lung cell, while functionalization and aspect ratio are the main influencing factors on the liver cell. ER stress is sensitive pathway for the size change, and oxidative stress is sensitive pathway for the functionalization. Overall, our approach shows that an AOP can be developed using existing data and BN model by identifying data gaps between the MIE, KEs, and the AO. Acknowledgement: This work was supported by a grant from the Korean Ministry of Environment through 'Environmental Health R&D Program' (2021003310005).

01.03.09 Adverse Outcome Pathways Using Japanese Medaka Embryos (Oryzias latipes) Exposed to 2,3,7,8-Tetrachlorodibenzodioxin

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Adverse outcome pathways (AOPs) are an open, collaborative framework that categorizes the impact of chemicals from the molecular to the ecosystem level. This research aims to refine two existing AOPs (21 and 150) that are initiated by dioxins and dioxin-like chemicals binding to the Aryl hydrocarbon receptor (AhR), ultimately resulting in an adverse outcome of altered cardiovascular development in a multitude of organisms. AOP 21 proposes that crosstalk between HIF-1a and AhR causes the adverse effect, while AOP 150 proposes that increased COX-2 is the key event downstream of AhR. Our preliminary studies show that medaka (Oryzias Latipes) embryos exposed for 1 hour to 0.001 ppb of dioxin during development display altered cardiovascular development seen as reduced blood flow, pericardial edema, and impaired angiogenesis. When embryonic development is adversely impacted by dioxins, we hypothesize that there are differences in gene and protein expression, which will distinguish the molecular level key events in these AOPs. Medaka embryos were exposed to 0.001, 0.01, 0.1, 1, and 10 ppb of 2,3,7,8-Tetrachlorodibenzodioxin (TCDD) for 1 hour at the 4-hour-post-fertilization mark. Embryos were collected at 2 days post fertilization (dpf) and 7dpf. We conducted non-targeted proteomics and qPCR on embryo homogenates, with primers targeting the AhR2a, HIF-1a, and COX-2 genes. These were linked to higher-level adverse effects observed as cardiac impairment via heart rate analysis using open-source *HeartBeat* software on 7dpf videos. We also recorded malformations, scoring by the severity of the pericardial edema. Our qPCR results strongly suggest AOP 21 is likely more influential than AOP 150 in causing altered cardiovascular development. Refining these AOPs will benefit society by improving our ability to respond to chemical contaminants of concern more effectively.

01.03.10 Leveraging Zebrafish As an In Vivo Model to Confirm the Molecular Initiating Event in Seizures Induced by Tetramethylenedisulfotetramine (TETS)

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Originally developed as a rodenticide, tetramethylenedisulfotetramine (TETS) causes life-threatening seizures. Despite a world-wide ban on its production, TETS is widely available on the black market and has been documented to cause numerous human deaths due to accidental or intentional exposures. Due to its stability in water and tissues, TETS is a persistent environmental contaminant. The proposed mechanism of action for TETS-induced seizures is antagonism of γ-aminobutyric acid type A receptors ($GABA_AR$). *In vitro* electrophysiological studies of transformed cells overexpressing specific $GABA_AR$ subunits identified the $GABA_AR$ α2β3γ2 subtype as the molecular target of TETS, but this has yet to be confirmed in a more physiologically relevant model system.Previous studies in our lab have established larval zebrafish as an *in vivo* model of TETS-induced seizures, and demonstrated that increased motility triggered by TETS correlated with both seizure behavior and electrical field activity in the optic tectum that resembled electrographic seizure activity. Here, we leveraged the larval zebrafish model to confirm the molecular initiating event (MIE) that leads to the adverse outcome of seizure events by comparing the efficacy of $GABA_AR$ subunit-selective positive allosteric modulators (PAMs) in reducing TETS-induced motility in larval zebrafish. We tested PAMs targeting α1, α2, α2/3/5, α6, ß2/3, ß1/2/3, and δ subunits and compared their efficacy to the non-selective benzodiazepine midazolam (MDZ). Our data demonstrate that PAMs targeting α2 and α6 (SB-205384 and SL-651,498, respectively) were effective at mitigating TETS-induced seizure behavior. Combinations of SB-205384 and MDZ or SL-651,498 and 2-261 (ß2/3 selective) effectively mitigated TETS-induced seizure behavior at concentrations that did not elicit sedating effects in a photomotor behavioral assay, whereas MDZ alone caused sedation at the concentration required to stop seizure behavior. These results further elucidate the MIE by which TETS induces seizures and provides insight regarding specific countermeasures against this chemical convulsant. Future experiments include the use of $GABA_AR$ subunit knock-out zebrafish to confirm the role of subunit selectivity in TETS toxicity. The methods presented are an example of a medium-high throughput "top-down" AOP development strategy, and could be translated to investigate the MIE of other pro-convulsants. This work is supported by NINDS (grant # U54 NS079202).

01.03.11 Identifying Potential Key Events Linking Xenoestrogen Exposures in Female Fathead Minnows to Reproductive Impairment

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Several adverse outcome pathways (AOPs) have linked endocrine related molecular initiating events like aromatase inhibition, androgen receptor (AR) agonism, and estrogen receptor (ER) antagonism, to reproductive impairment in adult (AOP25, AOP23, AOP30; aopwiki.com). It has been demonstrated that ER agonists can also lead to reproductive impairment in mature fish, however, the early key events (KEs) leading to this are mostly unknown. The aim of this study is to develop some hypotheses regarding the potential mechanisms through which exposure to exogenous ER agonists might lead to reproductive impairment in female fish. We exposed reproductively mature fathead minnows to low (1 ppt) and high (10 ppt) concentrations of 17α-ethinylestradiol (EE2), a strong ER agonist that is a common environmental pollutant, for 14 days. The response to EE2 was contrasted with that observed following a 14-day exposure to 17β-trenbolone (TRB) (500 ppt), a well-known AR agonist. Exposure to either high EE2 or TRB caused a decrease in plasma concentration of 17β-estradiol (E2) and ex vivo ovarian testosterone (T) production (albeit not significant in the case of high EE2). Exposure to high EE2 also caused a significant increase in plasma VTG and decrease in ex vivo ovarian E2 production. The cumulative eggs spawned/female over the experiment also decreased in both high EE2 and TRB treatments with a larger decrease in the TRB exposure. Based on these results and understanding of the hypothalamic–pituitary–gonadal axis (HPG axis), we hypothesize that ER agonism by EE2 causes negative feedback that results in decreased production of maturation inducing steroid leading to impaired oocyte maturation and ovulation, eventually resulting in a reduction in cumulative fecundity. Ovarian transcriptomic data from the experiment are being analyzed to evaluate consistency with our working hypothesis and/or provide insights into other potential key events based on Gene Set Enrichment Analysis and DAVID pathway enrichment analysis. *The contents this abstract neither constitute nor necessarily reflect USEPA policy.*

01.03.12 Quantification of an AOP Network for Effects of UV Radiation by Statistical and Bayesian Network Modelling

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An adverse outcome pathway (AOP) network has been developed to describe the adverse effect of UV-B radiation (AOP #327–330). This tentative AOP, which is the first AOP for a non-chemical stressor, is a complex network linking a molecular initiating event (MIE: cellular ROS formation) to an adverse outcome (AO: reduced survival of a crustacean), through eleven potential key events (KE). The AOP structure has been developed based on literature studies and experimental data, and quantified by experimental studies, where *Daphnia* individuals were exposed to UV radiation in 6 different dose-rates in the range 0 - 0.4 w/m2. All variables (the MIE, KEs, and AO) were measured with a minimum of three repeated measurements for each dose-rate. The resulting proposed AOP network consists of four potential pathways from the stressor to the adverse outcome, which has been quantified with equations fitted to the data using the EPA tool Benchmark Dose Analysis Software. The current study aims to further develop this quantitative AOP (qAOP) by a combination of statistical modelling and causal probabilistic modelling (Bayesian network), within the project RiskAOP (https://www.niva.no/en/projectweb/RiskAOP). Bayesian regression modelling is applied to quantify the key event relationships (KERs) as dose-response curves and the associated uncertainty based on experimental data. Additional statistical methods such as boosting and structural equation models will be explored to support the quantification of KERs. The fitted regression models are subsequently used to generate probability distributions for quantifying the KERs as conditional probability tables in the Bayesian network (BN) model. The fully quantified BN model can be used to further explore the properties of the qAOP. For example, sensitivity analysis of the BN-AOP model can be used for ranking the pathways of the AOP network according to the strength of their influence on the adverse outcomes. The BN-AOP can potentially be used both for prognostic inference (from the MIE to the AO) and vice versa for diagnostic inference (from the AO backwards via the KERs to the MIE). The approach demonstrated here – a combination of expert knowledge, experimental data, exploratory data analysis, and probabilistic modelling - is a promising strategy for the data-oriented selection of key AOPs and the identification of knowledge gaps associated with these AOPs.

01.04 Bioaccumulation Beyond Lipid-based Processes: Microplastics, Nanoparticles, and Emerging Contaminants

01.04.01 A Review of Bioaccumulation Patterns of Per- and Polyfluoroalkyl Substances (PFASs) in Marine Organisms

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Per- and polyfluoroalkyl substances (PFASs) 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. There are over 4000 compounds recognized under this contaminant category which complicates monitoring and risk assessment efforts. The objective of this summary is to compare patterns of PFAS accumulation in marine organisms and to identify chemicals of greatest potential concern. Here we present the accumulation patterns of perfluoroalkyl carboxylates (PFCAs) and perfluoroalkyl sulfonates (PFSAs), and their precursors, across marine mammals, birds, and fish in multiple tissue types and biological compartments. Despite differences in geographical and temporal scales, the longer-chain (≥C8) PFCAs were found to be dominant across different taxa. Perfluoroundecanoic acid (PFUnDA, C11) was particularly elevated across taxa as well as across temporal studies indicating its persistence and bioaccumulative potential. Perfluorooctanesulfonate (PFOS, C8) dominated the PFSA profiles in multiple tissues across taxa. Further, perfluorohexanesulfonate (PFHxS, C6) and perfluorodecanesulfonate (PFDS, C10) as well as precursors such as perfluorooctane sulfonamide (FOSA) were also detected in several marine organisms. Taxonomic and tissue-specific differences in accumulation were noted. For example, PFASs were observed to be elevated in protein-rich tissues and were notably high in bird eggs as well as in mammalian and avian livers. Blood (mammals and birds) and muscle (fish) concentrations are also presented. Overall, fish are reported to accumulate lower levels of PFASs per gram of wet weight than do birds and mammals. Fish data also present differences between bony and cartilaginous fishes. Further, the accumulation patterns indicate higher levels of PFASs in air-breathing organisms (seabirds and marine mammals) than in fish, suggesting differences in elimination kinetics. It must be noted that multiple environmental and biological variables including dietary, metabolic, and physiological differences could significantly affect observed PFAS burdens. Identification of PFAS accumulation patterns across habitats and taxa, such as presented in this summary, could be applied to determine high-risk populations, focus criteria development, risk assessment and monitoring efforts.

01.04.02 Bioaccumulation of Per- and Polyfluoroalkyl Substances (PFAS) Levels in Backfin Tuna (Thunnus atlanticus) at Biscayne Bay, Miami, Florida: A Food Safety Concern

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Per- and Polyfluoroalkyl substances (PFAS) especially legacy perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), have been reported in several environmental compartments, such as surface water, drinking water, wastewater, showing higher accumulation in aquatic organisms due to their proteinophilic characteristics. Although PFOA and PFOS are banned from production and use, alternative compounds are still poly- or perfluorinated and their widespread use and application has resulted in increased concentrations of emerging short-chain PFAS in the aquatic ecosystem. Florida is the "fishing capital of the world", and the US lead in the number of recreational anglers (36% of all US recreational fishing trips). Blackfin tuna (*Thunnus atlanticus*) is the most frequently consumed fish type in South Florida and mostly abundant during the spring and summer season (March-May). Bioaccumulation of PFAS, including legacy and emerging PFAS, in recreational fish can thus constitute a food safety concern. Biscayne Bay is a sub-tropical estuary located primarily in Miami-Dade County and a unique marine environment of national significance, due to its productive mangrove, seagrass and coral reef ecosystems, diverse natural resources and fishing opportunities; especially offshore of the southern barrier islands are hot spots for recreational fishers. After a massive fish kill at Biscayne Bay in 2020 associated with low oxygen levels, there is an urgent need to identify the input of anthropogenic contaminants of concern to help inform action plans and management strategies for water quality protection. In this study, we will determine PFAS concentrations in fish muscles and assess their bioaccumulation in blackfin tuna inhabiting Biscayne Bay. The human health risks associated with tuna fish consumption will be also evaluated. Black fin tuna muscles (N=20) will be screened for legacy and emerging PFAS collected from the Biscayne Bay using an alkaline extraction procedure, followed by a dispersive solid phase extraction (dSPE) cleanup using Florisil® and graphitized carbon black (GCB), and liquid chromatography- tandem mass spectrometry (LC-MS/MS) analysis. Surface water samples will be also collected where fish samples were taken. Waters will be processed by a previously developed and validated SPE method following LC-MS/MS.

01.04.03 Contamination by Per- and Polyfluoroalkyl Substances and Phthalate Esters in Oysters From Florida, United States

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Environmental chemical contamination is a major global safety issue that poses a serious threat to the marine ecosystem and to human health. Two of the most concerning groups of chemicals are composed of per- and polyfluoroalkyl substances (PFAS) and phthalate esters (PE). These compounds are widely used as commercial and consumer products, such as food packaging, lubricants, paper products, surfactants, plasticizers, pharmaceuticals, cosmetics, personal care, among others. Many of these compounds represent a threat to living organisms due to their biopersistency, bioaccumulation, and biomagnification in the food chain. Bivalve mollusks have been established as good environmental contamination sentinels due to their filter-feeding habits and limited mobility. Thus, the current study investigated PFAS and PE contamination in American oysters (*Crassostrea virginica*) sampled from three different locations in Florida, U.S.: Miami, Key West, and Tampa. Following sampling, all oysters (n = 118) were measured and weighed (shell weight, shell height, shell width, shell thickness, and animal weight). For the PFAS analysis, samples were extracted with a methanol and potassium hydroxide solution. Then, extracts underwent evaporation, cleanup, and filtering processes before being analyzed by liquid chromatography-tandem mass spectrometry. For the PE analysis, samples were extracted with methylene chloride, evaporated, and cleaned with florisil, followed by gas chromatography-mass spectrometry analysis. Data analyses were conducted using the Agilent MassHunter and Chromeleon software for the PFAS and PE data, respectively. A Condition Index (CI) based on oyster metrics and weight was also calculated to evaluate oyster physiological health status. Preliminary results indicate oyster contamination by both PFAS and PEs, which may indicate potential deleterious effects in these organisms. Variations in contaminant concentrations among the studied areas will be evaluated, which may reveal different levels of pollutant discharge in each of the sampling areas. Correlations between contaminant concentrations, locations, and CIs will also be investigated. Continued monitoring of these pollutant concentrations in bioindicator species like oysters is necessary to better understand the spatial and temporal variation of these contaminants. The findings might serve as proxies for decision-making related to risks to human health and environmental monitoring and management.

01.04.04 Legacy and Contaminants of Emerging Concern in Tree Swallows Nesting in Three Drainages of the Milwaukee Estuary, WI

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There are often little data for biota, either on exposure to or effects from emerging contaminants (CECs). This Great Lakes Restoration Initiative (GLRI) project provides new data on CECs, as well as, legacy contaminants for a model species, the tree swallow (*Tachycineta bicolor*). Exposure to multiple classes of emerging contaminants were assessed in tree swallow tissues and their food from birds nesting along three drainages, with differing land use patterns, that comprise the Milwaukee Estuary, WI. Samples were also collected from a remote lake in northern Wisconsin for comparison. Exposure to the bioaccumulative contaminants, such as PCBs, PBDEs, and PFAS were generally below concentrations that have been linked with reproductive effects or physiological responses. Only a few pharmaceutical and personal care products (PPCPs) and non-organochlorine pesticides (N-OCs) were detected, as was expected, and included DEET and Iopamidol, but also amphetamines and azithromycin for PPCPs, and atrazine and desethylatrazine for the N-OC pesticides. Desethylatrazine was found in most samples (eggs and nestling carcasses), but its parent compound, atrazine, was only rarely detected. The PPCPs, when detected, were more likely to be detected in liver rather than egg tissue. Both DEET and amphetamines were also detected in the remote lake which probably reflects the wide-spread use of DEET as an insect repellent. Concentrations of polycyclic aromatic hydrocarbons (PAHs) in tree swallow diet samples were higher in all three drainages compared to the reference lake, however, within the Milwaukee Estuary, the Menomonee River was 10 times more contaminated with PAHs than the other two drainages in the estuary. Associations of biomarker assessments with contaminant exposure, both legacy and emerging contaminants, will be presented. Sediment, the only abiotic matrix sample, was not necessarily predictive of exposure in birds.

01.04.05 Bioaccumulation of Per- and Polyfluoroalkyl Substances (PFAS) in Freshwater Fish From the Great Lakes Region

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Per- and polyfluoroalkyl substances (PFAS) are synthetic organic compounds that have been manufactured since the 1940s for use in non-stick coatings, waterproof fabrics and carpets, firefighting foams, food packaging and many other applications. PFAS are highly mobile and resistant to degradation. One PFAS of particular concern for environmental health is perfluorooctane sulfonate (PFOS). PFOS has been detected globally in water, sediment, fish, and wildlife and is highly bioaccumulative in aquatic species. Epidemiological studies have linked widespread PFOS exposure in people to several health effects. Thus, consumption of PFOS-contaminated fish is an exposure route of great concern when considering risks to people, especially subpopulations of people that regularly consume freshwater, caught-fish. Accordingly, to better understand bioaccumulation and provide a scientific basis for water quality regulations and fish consumption guidance, PFAS monitoring programs in the states of Minnesota and Wisconsin have prioritized paired fish and water sample collections. We will provide an overview of the bioaccumulation patterns of PFOS and other PFAS compounds in a diverse array of freshwater fish species from over 50 waterways across Minnesota and Wisconsin. Temporal and spatial trends will be examined, as will differences in bioaccumulation among, and variability within, species and waterbody types. In addition, we will describe the different approaches used by Minnesota and Wisconsin to calculate statewide bioaccumulation factors (BAFs) for the purpose of deriving water quality standards.

01.04.06 Understanding the Impact of Per- and Polyfluoroalkyl Substance Contamination in Laboratory Materials and Model Organisms on Laboratory-Based Studies

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Per- and polyfluoroalkyl substances (PFAS) are persistent chemicals that present risks to public health and the environment. The ubiquitous nature of this class of compounds has the potential to impact laboratory based exposure and toxicology experiments through PFAS contamination of common aquatic culture materials. Results from laboratory exposure experiments could be easily confounded if additional, unknown PFAS sources remain unaccounted. This study investigated the PFAS contamination in common fish feed, habitat materials, and Pimephales promelas (fathead minnows) from several aquaculture farms that are used in fisheries management and in laboratory experiments to study PFAS bioaccumulation. Results suggest that PFAS contamination is present in common fish feed ranging from 0.13 ng/g to 29.14 ng/g and can also be found in aquaculture minnows at concentrations ranging from 0.05 ng/g to 273.88 ng/g. Concentrations of a suite of PFAS present in each sample type will be presented along with recommendations for developing laboratory exposure experiments with the least PFAS contamination. Considering many ecological and human health recommendations are based on bioaccumulation and toxicity models created using aquatic organisms, unaccounted PFAS contamination in these systems could confound these bioaccumulation and toxicity results. Understanding the sources of PFAS contamination to laboratory exposure experiments using model organisms represent an important initial step to support more risk-based decisions at contaminated sites.

01.04.08 Proposing a Bioaccumulation Metric Criteria Framework for Plastic Particles in Marine Biota and Foodwebs

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Bioaccumulation science is of paramount importance to assess and understand the bioaccumulation behaviour and biomagnification capabilities of pollutants in marine organisms and foodwebs exposed to abiotic concentrations. Ocean pollution by ubiquitous plastics particles such as plastic nanoparticles (nanoplastics size ranging from 1 nm up to 1000 nm) and microplastics (particle size < 5mm) is one the most pervasive ecological footprints by humans in the Anthropocene due to the accelerated rate of plastic production, emissions, and associated health impacts in the oceans. Understanding the bioaccumulation potential of microplastics is critical to assessing the foodweb bioaccumulation and biomagnification capabilities of these emerging contaminants and addressing fundamental research gaps regarding the microplastic kinetics as a function of exposure, ingestion, net accumulation, and elimination of microplastics. While bioaccumulation and biomagnification are key criteria part of the risk evaluation of chemicals under the Stockholm Convention of POPs, as well as within the national policies for pollutant management and priority toxic chemicals aimed to protect public and environmental health, limited research, and an ongoing debate exists as to whether microplastics bioaccumulate or/and biomagnify in marine ecosystems and foodwebs. Thus, a concerted bioaccumulation assessment framework for nanoplastics and microplastics is urgently needed to address fundamental research gaps concerning the foodweb bioaccumulation and biomagnification of these plastics particles. A framework for screening primary and secondary microplastics as bioaccumulative pollutants has yet to be developed. Here, a proposed bioaccumulation framework is developed as an approach integrating bioaccumulation and biomagnification metric criteria, including the Concentration Factor (CF) or Bioconcentration Factor (BCF); the Accumulation Factor (AF) or Bioaccumulation Factor (BAF); Biota-Sediment Accumulation Factor (BSAF); the Magnification Factor (BF) or Biomagnification Factor (BMF); the predator-prey Biomagnification Factor (BMF_{TL}); and, Foodweb Magnification Factor (FWMF) or apparent Trophic Magnification Factor (TMF). The proposed framework can support monitoring strategies to assess the bioaccumulation potential of microplastics in marine organisms and support ecotoxicological risk assessment to inform waste management policies for microplastics as global emerging pollutants of great concern.

01.04.10 Bioaccumulation and Biomagnification of Perfluoroalkyl Substances in the Subarctic Ringed Seal Food Web of Lake Melville, Northern Labrador, Canada

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Perfluoroalkyl substances (PFAS) are a series of synthetic fluorinated organic compounds that include perfluoroalkyl acids that are highly persistent. Some PFAS possess high bioaccumulation potential. The ringed seal (*Phoca hispida*) food web of Lake Melville, a semi-enclosed estuarine fjard located in northern Labrador of eastern Canada, may be impacted by these chemicals. The Lake Melville region (53° 41’N 59° 43’ W) is currently undergoing environmental changes including climate warming, flooding, and reservoir creation through clear-cutting 400,000 m^3 of timber for hydroelectric power developments. This hydroelectric project took place at Muskrat Falls, located on the lower Churchill River. This river drains into the Lake Melville estuary. We hypothesize the perturbations associated with the hydroelectric development may increase contaminants such as PFAS in Lake Melville. Bioaccumulating contaminants are relevant to the ringed seal food web and are of consequence for local Inuit populations for which ringed seals, fish, and invertebrates are important traditional country foods. In this study, a total of 57 biota samples were collected to investigate concentrations, congener profiles, bioaccumulation, and biomagnification of PFAS throughout the ringed seal food web. Samples of invertebrates, fish and seal livers and muscles were chemically extracted and then analyzed for 17 PFAS using ultra-high performance liquid chromatography coupled with tandem mass spectrometry. PFAS are ubiquitous in Lake Melville biota. Long-chain odd numbered perfluorocarboxylic acids (PFCA) and perfluorooctane sulfonate (PFOS) were the major congeners in livers of fish and seals. Fish residing close to riverine and terrestrial PFAS sources and seals that exhibited near-shore feeding strategies were associated with higher PFAS levels. Two ringed seal food webs, brackish and marine, can be delineated in Lake Melville. Trophic biomagnification factors (TMF) indicate that long chain PFCA, PFHxS, and PFOS biomagnify throughout these ringed seal food webs. Bioaccumulation and biomagnification of PFAS in the ringed seal food web is consistent with previously reported serum protein-binding that shows maximum binding and accumulation of PFAS with 8-9 carbons. The PFAS congener pattern in Lake Melville ringed seals are consistent with other areas in the Arctic and sub-Arctic receiving atmospheric inputs. This study is the first to investigate PFAS in a complete food web in Labrador.

01.04.11 Bioaccumulation of PFOS in the Benthic Freshwater Amphipod Hyalella azteca

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The bioconcentration test with the freshwater amphipod *Hyalella azteca* (HYBIT) was recently suggested as alternative test system for bioconcentration studies. About 20 organic compounds with different hydrophobic character (log Kow 2.4-7.8) have been tested. The bioaccumulation potential of compounds is commonly expressed in the form of bioconcentration factors (BCF) determined in flow-through studies with fish according to OECD 305. Bioconcentration studies with *H. azteca* resulted in BCF estimates which show a strong correlation with fish BCF values. So far, only lipid accumulating substances have been tested with *H. azteca*. Further studies are required to elucidate the bioconcentration of non-lipid accumulating substances. PFOS was chosen as test item due to the ionic character as well as the environmental and regulatory relevance. PFOS, used since the 1940s, is a global pollutant and a representative of fluorochemicals that can be found in nearly all biota samples from nearly all places around the world. In this study several Hyalella BCF tests with PFOS were carried out under semi-static conditions. Different exposure concentrations were compared. Studies were carried out with male animals and the derived BCF values compared with the results from further studies with female animals carried out under comparable conditions. The test design enabled constant exposure conditions. Measurement of tissue concentrations in animal samples collected during the studies allowed to calculate BCF_{SS} and BCF_{kin} values. The results are compared with BCF values described in the literature.

01.04.12 Testing Strategy for Bioaccumulation Assessment of Nanomaterials Using Freshwater Invertebrate Species

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The high production volume of ENMs may lead to high pressure on the environment and a scientific assessment of ENMs that bioaccumulate in organisms and biomagnify in food webs is necessary. Within the regulation of chemicals in several jurisdictions, such as the European regulation REACH, the bioconcentration factor is the standard endpoint. The bioconcentration factor is mostly determined by flow-through fish tests. Several risk assessment regulations allow the usage of data gained during tests using invertebrates and such data may allow a waiver of futher tests using vertebrates. A literature study has elucidated the potential of different aquatic invertebrate species to be used in laboratory bioaccumulation studies on ENMs (Kuehr et al. 2020). Amphipods were identified as the most promising species for ENM testing. Modified BCF and BMF values can be calculated that fulfil the requirements of endpoints needed for the bioaccumulation assessment of ENMs under regulations like REACH. A testing strategy representing a modified version of the tiered approach presented by Handy et al. (2018), was recently proposed including an assessment scheme with the aim of defining a “bioaccumulative” or “non bioaccumulative” grading for ENMs without using fish. In addition, decision criteria / endpoints that are more robust regarding the analytical challenges involved in ENM studies compared to the established endpoints were suggested (Kühr et a. 2020). The poster will provide a summary of the tiered approach and describe the gaps and data requirements necessary to allow the implementation of the testing strategy using amphipods in the regulatory bioaccumulation assessment of ENMs.

01.04.13 Bioaccumulation Assessment of Micro and Nano Plastics in Bivalves Using Fluorescent Dyed Particles

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A vast amount of polymer based plastics are produced for our daily needs and end up as waste sooner or later. Plastic waste stored in landfills, or being released to the terrestrial environment, the sea or fresh water ecosystems can disaggregate and form smaller fragments in a size range between 100 nm to 5 µm, called microplastics (MPs) as well as particles with sizes below 100 nm, called nanoplastics (NPs). Tiny plastic particles are also purposely produced, e.g. for use in a wide range of personal care products. Following the use of these products the manufactured MPs (mMPs) reach the waste water, which is described as being one of the main sources of MPs in the aquatic environment. There are still some obstacles to overcome before a regulatory assessment of mMPs based on laboratory studies is possible. One of these obstacles is the lack of suitable methods for the quantification of exposure concentrations of the mostly carbon-only based polymers. Furthermore, investigations on the bioavailability, defined as the incorporation of particles into the tissue/ cells in contrast to the simple ingestion of the material are difficult to carry out as previously discussed for nanomaterials. Laboratory studies with invertebrates such as mussels and amphipods have been highlighted as a promising approach to support the regulatory bioaccumulation assessment of MPs and NPs. In this study, the freshwater bivalve *Corbicula fluminea* was used in laboratory studies to estimate the suitability of mussels to evaluate the bioavailability and bioaccumulation of NPs and MPs. We used fluorescence labelled polystyrene spheres of two different sizes, representing NPs and MPs to allow the quantification of the test items in the animal tissues. The laboratory studies included exposure and depuration phases which allowed the generation of data on the uptake and elimination kinetics of the particles by measuring the relative fluorescence as an indirect measurement of the test item burden in the bivalves soft tissue. Furthermore, isolated tissue compartments were dissected and analyzed to gain further insights into the bioavailability and fate of the particles. The excreted feces were analyzed as well. The preliminary laboratory investigations showed that filter-feeding bivalves are suitable to examine the bioavailability and accumulation of nPS and mPS and the data gained may be useful for the regulatory bioaccumulation assessment of micro- and nano- particulate compounds.

01.05 Complex Mixture Ecotoxicity: Novel Strategies and Techniques for Characterizing and Prediction

01.05.01 Prioritization of Contaminants of Emerging Concern in Puget Sound Using Novel Toxicological Measures: The Information Gained When Chemical Mixtures Are Considered

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Hundreds of chemicals are routinely detected in the marine waters and biota of Puget Sound. Many of these are considered emerging chemicals and are poorly characterized with respect to their occurrence, concentration, and toxicity, while others are well characterized and regulated. In previous work, we identified priority chemicals of emerging concern based on monitoring data that was compiled opportunistically from several monitoring campaigns in Puget Sound. Our prioritization approach generated Exposure Activity Ratios (EARs) by comparing that concentration data to biological responses from high throughput in vitro assays available from programs such as Tox21. Although these in vitro assays use human cells, receptors, proteins, DNA, and RNA, there is growing consideration of how to use these human-based in vitro assays for ecological receptors. One important advantage of using the in vitro assays is that they can inform a mechanistic understanding of toxicity and are therefore well suited for assessing chemical mixtures. In this work, we consider how our prioritization of PAHs and chemicals of emerging concern, such as pharmaceuticals and personal care products (PPCPs), plasticizers, and various chemicals present in consumer products, changes when the chemical mixtures are modeled. Our work relies on the ToxMixtures R package to sum EARs for chemicals detected in Puget Sound waters and biota with the same endpoints or AOP. We will discuss how and why our chemical prioritization changed while considering the management implications of our approaches.

01.05.02 Combined Effects of Chemical Mixtures Are Predictable for the Whole Transcriptome - a Proof of Concept Study With Zebrafish Embryos

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Humans and environmental organisms are constantly exposed to complex mixtures of chemicals. Extending our knowledge about the combined effects of chemicals is thus essential for assessing the potential consequences of these exposures. In this context, comprehensive molecular readouts as retrieved by omics techniques are advancing our understanding of the diversity of effects upon chemical exposure. This is especially true for effects induced by chemical concentrations that do not instantaneously lead to mortality, as is commonly the case for environmental exposures. However, omics profiles induced by chemical exposures have rarely been systematically considered in mixture contexts.In this study, we aimed to investigate the predictability of chemical mixture effects on the whole-transcriptome scale.We predicted and measured the toxicogenomic effects of a synthetic mixture on zebrafish embryos. The mixture contained the compounds diuron, diclofenac, and naproxen. To predict concentration- and time-resolved whole-transcriptome responses to the mixture exposure, we adopted the mixture concept of concentration addition. Predictions were based on the transcriptome profiles obtained for the individual mixture components in a previous study. Finally, concentration- and time-resolved mixture exposures and subsequent toxicogenomic measurements were performed and the results were compared with the predictions.This comparison of the predictions with the observations showed that the concept of concentration addition provided reasonable estimates for the effects induced by the mixture exposure on the whole transcriptome. Although nonadditive effects were observed only occasionally, combined, that is, multicomponent-driven, effects were found for mixture components with anticipated similar, as well as dissimilar, modes of action.Overall, this study demonstrates that using a concentration- and time-resolved approach, the occurrence and size of combined effects of chemicals may be predicted at the whole-transcriptome scale. This allows improving effect assessment of mixture exposures on the molecular scale that might not only be of relevance in terms of risk assessment but also for pharmacological applications. The study is published in EHP: https://doi.org/10.1289/EHP7773.

01.05.03 Understanding Frequency and Relevance of Non-Additive Effects of Pesticide Mixtures to Honeybees

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The toxicity of pesticide mixtures to honeybees has been a concern for decades. When assessing potential effects of mixtures, the pharmacological principle of additivity defines default expected effects of exposure to multiple chemicals. However, deviations from the additivity expectation may occur, namely greater than additive (synergistic) or less than additive (antagonistic) effects. The frequency and relevance of synergistic effects of pesticide combinations to pollinators is highly debated. An important consideration in such debate is how synergy is measured, and what a relevant numerical deviation from the additivity expectation should be considered "synergy". There is a lack of appropriate criteria for defining what a relevant deviation from additivity is for bees, and what is the frequency of such deviations among relevant pesticide combinations. To address these important data gaps we investigated a large Bayer database of bee acute toxicity data for binary to quaternary combinations of insecticides and fungicides that includes most relevant current use chemical classes, such as pyrethroids, neonicotinoids, fungicides, and many others. We computed additivity predictions for these combinations based on single chemical data according to the Concentration Addition (CA) model and compared these predictions to the observed experimental values via model deviation ratios (MDRs). In addition, we investigated a large dataset of bee toxicity data from the USEPA Knowledge base to study the intrinsic acute bee testing variability and used this information to propose MDR thresholds for defining relevant numerical thresholds to define relevant departures from additivity in honey bee testing. Our data suggest that the prevalence of greater than additive effects in pesticide combinations to bees in acute toxicity tests is very low (comparable to the frequency found for more extensively studied taxa groups, such as aquatic organisms). This work contributes to a better understanding of the prevalence and relevance of non-additive effects of pesticide mixtures to honeybees.

01.05.04 Addressing the Prediction of Mixture Effects Over Time With Toxicokinetics-Toxicodynamics Models of Survival

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Toxicokinetics-Toxicodynamics (TKTD) models are increasingly used for inference of toxicity indices of interest in Environmental Risk Assessment (ERA) thanks to their clear description of numerous mechanisms, from the kinetics of compounds inside organisms (Toxicokinetics, TK) to their related damages and effect dynamics at the individual level (Toxicodynamics, TD). TKTD models offer the advantage of accounting for temporal aspects of both exposure and toxicity, considering data points all along the time course of experiments. In addition, TKTD models allow predictions under untested situations from time-variable exposure profiles either measured in the field or simulated when evaluating risk assessment scenarios. Although ERA follows a compound-by-compound approach, in practice, living organisms are exposed to many chemical products, from agricultural, industrial and domestic sources. Using TKTD models to describe such mixture effects over time requires making assumptions a priori on potential interactions of involved products. These assumptions are then tested and evaluated based on fitting TKTD models to observed data under exposure to mixtures. This talk will illustrate how high-performance computing and machine learning may be of particular help for the inference of TKTD models without a priori knowledge to make it emerge the chemical interactions behind cocktail effects on life history traits.

01.05.06 Urban Pesticide Mixtures in Small Urban Streams Across the United States: Is There an Urban Pesticide Signature?

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Pesticide mixtures occur in urban streams globally, but the relation of occurrence to urbanization can be obscured by regional differences. In studies of five regions of the United States, we investigated the occurrence and potential toxicity of dissolved individual pesticides and pesticide mixtures in small urban streams. We analyzed 225 pesticide compounds in weekly discrete water samples collected during 6–12 weeks from 271 wadable streams; development in these basins ranged from undeveloped to highly urbanized. Sixteen dissolved pesticides were consistently detected in 16 urban centers across the five regions—we propose that these pesticides comprise a suite of urban signature pesticides (USP) that all are common in small U.S. urban streams. The USPs accounted for the majority of summed maximum pesticide concentrations at urban sites. Over 1.91 million unique mixtures of pesticide active ingredients were identified at urban sites, each containing between 2 and 17 compounds. The USPs comprised 83% of pesticides in the 20 most frequently occurring 2-compound unique mixtures at urban sites, of which carbendazim+prometon was the most common. USP concentrations, mixture complexity, and potential toxicity increased with the degree of urbanization in the basin. Basin urbanization explained the most variability in multivariate distance-based models of pesticide profiles, with region always secondary in importance. Although USPs were consistently detected in all regions, detection frequencies and concentrations varied by region, conferring differences in potential aquatic toxicity. USPs dominated the summed aquatic-life benchmark quotients for both fish and invertebrates, but not plants. Potential toxicity was highest for invertebrates (benchmarks were exceeded in 51% of urban streams), due most often to the neonicotinoid insecticide imidacloprid and secondarily to organophosphate insecticides and fipronil. Benchmarks were rarely exceeded in urban streams for plants (at 3% of sites) or fish (< 1%). Based on occurrence and contribution to potential toxicity, we propose that unique mixtures containing the USPs imidacloprid, fipronil, and carbendazim are priority candidates for mixtures toxicity testing.

01.05.08 Assessing Estrogenic Activity in Environmental Settings: Comparison of Analytical, In Vitro, and In Vivo Methods

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Endocrine disruption in organisms exposed to mixtures of steroidal estrogens and their mimics has been documented in aquatic ecosystems worldwide. Assessing the estrogenic activity of an environmental sample remains complicated by the multitude of available methods and their inherent limitations. Preliminary analyses utilized a matrix of analytical and biological data gathered from water samples at 370 stream sites and biological testing at a subset of these sites between 2010 and 2019 as part of the Great Lakes Restoration Initiative to address three questions: (i) Do different approaches to determine estrogenic activity provide similar results? (ii) Do methods to detect estrogenic activity differ in sensitivity in environmental samples? (iii) How prevalent is estrogenic activity across US Great Lakes Tributaries? Analytical chemistry data were reduced to compounds demonstrating estrogenic activity in seven ToxCast assays. Two analytical approaches were used to estimate estrogenicity: first, exposure-activity ratios (EARs) used to compare bioactivity of ToxCast assays related to estrogenic response with water chemistry were calculated for these compounds, and second, published estrogenic equivalency factors for estrogenic chemicals were used to compute 17β-estradiol equivalency quotients (EEQ), and summed (total EEQ). In addition, empirical techniques were used to estimate estrogenicity in water samples. Cell assays were used to estimate estrogenic activity in environmental water samples and normalized to 17β-estradiol activity (in vitro approach). Biological data for male fish exposed to environmental samples were reduced to those endpoints directly associated with estrogenic activity and included changes in gene expression and presence of vitellogenin in fish plasma (in vivo approach). Empirical data sets were normalized to laboratory controls for each individual experiment. Preliminary results uncovered limitations for each method, including limitations in the number of chemicals analyzed, cytotoxicity in cell-based assays, and intra-treatment variability of molecular and physiological endpoints. Few environmental samples exceeded a total estrogenic activity of 2 ng/L EEQ, a concentration likely to result in endocrine disruption in chronically exposed organisms. *The contents of this presentation neither constitute, nor necessarily reflect, USEPA policy.*

01.05.10 Using the New R Package "ToxMixtures" to Evaluate Potential Biological Effects of Pesticide Mixtures in Great Lakes Tributaries

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Humans create and use hundreds of thousands of chemicals, leading to the widespread presence of diverse chemical mixtures in aquatic ecosystems that can be difficult to assess for ecotoxicity. Here, we present an R package—ToxMixtures—to analyze, visualize, and interpret measured concentration data for individual chemical compounds and mixtures of chemicals identified in the same sample as they relate to chemical potency across multiple biological targets. ToxMixtures combines outputs from the R-package toxEval (http://usgs-r.github.io/toxEval/index.html), the ToxCast high through put screening database, and multiple open access sources related to molecular biology and adverse outcomes to provide information on the types of biological systems potentially affected by contaminants. Users can aggregate chemical concentration and potency information across ToxCast assays that are connected to gene induction/inhibition, biological pathways, and adverse outcomes to assess the combined influence of the chemical mixture in each sample. We demonstrate the utility of ToxMixtures to prioritize pesticides and their mixtures detected in Great Lakes tributaries. Chemical mixtures observed here were linked to multiple biological processes including xenobiotic metabolism, oxidative stress, cell signaling, and endocrine function, as well as adverse outcome pathways describing reproductive disfunction and population declines of sensitive species. Many of the herbicides detected in tributary samples share bioactivity in assays targeting common protein targets (e.g., cytochromes P450), suggesting shared cellular responses and the potential for additive effects by multiple compounds co-occurring in watersheds with high pesticide use. The package leverages a wealth of knowledge about the in vitro responses of individual chemicals together with the types of biological systems possibly influenced by the chemicals detected in environmental samples. Using this tool, researchers/risk assessors can organize discussions about the potential biological effects of chemicals and mixtures in water samples, which can guide additional hypothesis-driven monitoring and experimentation. The contents of this abstract neither constitute, nor necessarily reflect, USEPA policy.

01.05.13 Effects of Environmental Contaminants of Emerging Concern Mixture on In-Situ Fish Health

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The sustainability of fish population is a rising issue due to the pollutants in runoff that are washed into areas such as tributaries that feed the Great Lakes. The pollutants contain contaminants of emerging concern (CECs), including a diverse range of industrial compounds, personal care products, pharmaceuticals, and naturally produced steroid hormones. These CECs can interact with the cellular pathways or metabolic processes that can lead to issues with fish behavior, physiology, and morphology. The objective of this research is to identify CECs mixtures that pose a risk to fish population health due to their presence and concentrations in the Great Lakes tributaries. Through recent sampling of water, sediment, and fish tissue in the Great Lakes watershed, the presence of CECs is well established. Pairs of mature fathead minnows (*Pimphales promelas*) were exposed for 21 days to waters from nine field sites in Grand Rapids, MI, through a daily 50% renewal static exposure. A range of biological endpoints including physiological markers of maturity (steroid hormone concentrations), indicators of estrogenic endocrine disruption (plasma vitellogenin concentrations), and apical endpoints (behavior, fecundity) were assessed during and following the exposure. In total, we analyzed 332 plasma samples from this exposure, with at least 30 samples from each of the nine treatments. Biological endpoints across sampled field sites were compared with water chemistry to identify sites and CEC mixtures detrimental to fish health. The results show exposure to water from several field sites altered fecundity. Changes in fecundity were also correlated with changes in plasma vitellogenin, estradiol, and testosterone. These results highlight the continued threat CECs pose to fish population sustainability and can assist natural resources managers in planning conservation efforts.

01.05.15 Disruption of Thyroid Hormone Signaling and Other Endocrine Pathways by Headwater Pollutant Mixtures

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Thyroid hormone signaling (THS) plays a crucial role in metabolism, energy expenditure, growth, and development in vertebrates. The thyroid hormone system can be disrupted at various levels including thyroid hormone biosynthesis and secretion, blood and transmembrane transport, metabolism and local actions of thyroid hormones. Nevertheless, there is little information regarding the potential of environmental pollutant mixtures to interfere with THS. In the present study, water samples from a headwater in South Bohemia, Czech Republic, were collected with two types of passive samplers covering a wide range of dissolved organic micropollutants. The sampling sites were situated upstream and downstream of a municipal wastewater treatment plant (WWTP) effluent to determine its impact on both bioactivity and chemical composition. *In vitro* cell-based bioassays were applied to assess (ant)agonism to thyroid receptor (TR) and transthyretin (TTR) binding potencies of sample extracts. In addition, retinoid-like, dioxin-like, (anti-)estrogenic and (anti) androgenic activities were tested to gain a broad profile of endocrine disruptive potentials. Thyroid receptor and TTR binding potencies were detected and increase of both THS-disruptive effects was observed downstream of the WWTP effluent. Besides, the sample extracts elicited androgenic, dioxin-like, estrogenic and anti-estrogenic activities, while there was no effect on signaling of retinoic acid receptor. Multi-residue analysis based on liquid chromatography with tandem mass spectrometry (LC-MS/MS) targeted 102 compounds, with 77 compounds detected at least once. The highest concentrations were determined for telmisartan, iopromide, diclofenac, lamotrigine, tramadol and metoprolol. The cumulative concentrations of target compounds showed a clear impact of the WWTP effluent, which is in line with the increase of the detected endocrine disruptive potencies. Our study presents novel findings regarding disruptive potential of surface water samples on multiple levels of thyroid signaling highly relevant for aquatic biota and human health. It aims to identify the driver compounds in the follow-up steps by linking the observed effects with the detected chemicals using their relative effect potencies (REPs), as well as effect directed analysis (EDA). The work is supported by the Czech Science Foundation Grant No. 20-04676X.

01.05.16 Evaluation of Complex Mixture Toxicity: An Effects-Driven Analysis in the Milwaukee Estuary Area of Concern (Milwaukee, WI)

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Lakes Toxicology & Ecology Division; C. Schaupp, ORISE Post-doctoral Research Participant, United States Environmental Protection Agency / Great Lakes Toxicology and Ecology Division; D.L. Villeneuve, U.S. Environmental Protection Agency / Center for Computational Toxicology and Exposure

Anthropogenic activities (e.g., urbanization, agriculture, industrial operations) result in complex contaminant mixtures in associated watersheds. To protect organisms inhabiting these aquatic environments, it is important to account for mixture toxicity during ecotoxicological effects assessment. Here, we present several different strategies to evaluate complex mixture toxicity and identify individual constituents, contaminant groups, and mixtures of greatest concern within the Milwaukee Estuary Area of Concern (AOC; Milwaukee, WI). To evaluate potential *in vivo* effects of contaminant mixtures, adult fathead minnows (*Pimephales promelas*) were caged at 8-11 study sites across the AOC in 2017 and 2018. Following 96-h of exposure, fish tissues were sampled and used for targeted effects analyses (e.g., sex steroid concentrations, gene expression). Concurrently, composite water samples were collected and analyzed for nutrients and 178 wastewater indicators and pharmaceuticals, and characterized for *in vitro* bioactivity that might lead to adverse effects (e.g., endocrine related and pathway-specific effects; Attagene cis- and trans-Factorial and T47D-Kbluc assays). Chemicals were subsequently grouped based on putative mode of action (MOA), and susceptibility of local fish species to potential adverse effects mediated through chemical interactions with specific proteins using the USEPA SeqAPASS tool and data from the Map of Life Project. Individual constituents, chemical groups, and mixtures potentially contributing to integrated biological effects were identified using multiple lines of evidence including *occurrence at impacted sites* (qualitative analysis), *contribution to cumulative toxic units* (maximum cumulative ratio; ECOTOXicology Knowledgebase, ToxCast database), and *covariance with measured effects* (randomForest regression). Overall, this study demonstrates multiple strategies that can be employed to evaluate complex mixture toxicity, and highlights the utility of integrating chemical, biological, and computational techniques for cumulative effect analysis. *The contents of this abstract neither constitute nor necessarily reflect USEPA policy.*

01.05.17 Role of Alkylated Polycyclic Aromatic Hydrocarbons in Mixture Toxicity From a Legacy Creosote Site

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Creosote is a wood treatment product, often derived from distillation of tars. However, as a complex mixture containing mostly polycyclic aromatic hydrocarbons (PAHs) and their derivatives, the toxic effects of legacy creosote contamination are poorly understood. In particular, alkylated PAHs are products of PAH weathering and are known to be abundant constituents of creosote and many petroleum products. 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. The goal of this study is to understand the contribution of alkylated PAHs to the toxicity of a complex 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 (GC-MS/MS) for over 60 alkylated and parent PAHs. To assess toxicity, embryonic zebrafish are exposed to PAH mixtures in 96-well plates and are observed at 24 and 120 hours post fertilization for a suite of behavioral and morphological endpoints. Zebrafish embryos are exposed to both passive sampling extracts from the contaminated site and representative mixtures from reference materials in order to quantitatively describe the contribution of alkylated PAHs to the toxicity of a complex mixture of weathered PAHs. Chemical analysis of the passive sampler extracts has shown that alkylated PAHs are far more abundant than parent PAHs. Additionally, results show that representative mixtures composed of the most abundant parent and alkyl PAHs failed to replicate the toxicity of whole passive sampler extracts. This suggests that less abundant or unidentified compounds may play an important role in mixture toxicity. Mixture interactions between PAHs appear to mediate the observed toxicity in zebrafish but further work is necessary to confirm or refute these preliminary findings. Ongoing work seeks to determine the drivers of toxicity and the role of alkylated PAHs by fractionating the passive sampler extracts 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.

01.05.18 Evaluation of the Interactions and Gene Expression of Antioxidant Enzymes in Cyprinus Carpio Embryos Exposed to Aluminum, Penicillin, Metformin and the Mixture

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In the aquatic environment, organisms are exposed to complex mixtures of pollutants, which could alter the toxicity of each compound. Drugs such as penicillin and metformin, and metals such as aluminum are some of the pollutants that have been found mixed in water bodies. These substances are known to be capable of generating an oxidizing environment resulting in damage to biomolecules. However, the few studies that analyze its toxicity in aquatic species have been based on individual exposure experiments, so the present study evaluates the type of interaction produced on *Cyprinus carpio* embryos exposed to binary and tertiary mixtures of penicillin, metformin and aluminum at concentrations corresponding to NOAEL and LC_{50}, obtained from an acute toxicity study in which the number of dead and alive embryos was quantified at 96 hours of post-fertilization exposure. For the determination of the type of interaction in the pollutant mixtures, the observed effect was lethality. Dilutions were made from the NOAEL and LC_{50} and the organisms were exposed up to 96 hpf. After counting the living and the dead, the results obtained were entered into the CompuSyn software, which determines the value of the Combination Index that indicates the type of interaction in the mixtures. Sublethal toxicity was studied by evaluating the gene expression of SOD, CAT and GPx at 12, 24, 48 and 72 hours in embryos exposed to the individual substances and the tertiary mixture in concentrations corresponding to the NOAEL (where no effect of lethality is found) using RT-PCR. The results show that in the binary mixtures at low concentrations, the type of interaction that is predominantly observed is synergism, while in the tertiary mixture antagonism is observed. Gene expression changes over time, although the group of embryos exposed to the tertiary mixture of pollutants shows the highest increase during the first half of the exposure. It is concluded that individually, aluminum was the substance with the highest acute toxicity, followed by metformin and finally penicillin. The interactions present in the mixtures modify the observed effect while the individual substances and together produce changes in gene expression with respect to the reference gene.

01.05.19 Toxicity Screening of Individual Chemical Compounds in DPM Using ToxCast Assay Data - Based Prediction Models and CTD

S. Bea, J. Choi, University of Seoul / Environmental Engineering

Diesel Particulate Matter (DPM) refers to all particulates except exhaust gas among by-products generated during the combustion process of fuel in a diesel type internal combustion engine. According to research, DPM cause human respiratory health effects, neuroinflammation and impaired the reproductive functions. The characteristic of DPM is that the composition of DPM changes according to the process of generation, temperature, and composition ratio of fuel, and for this reason, toxicity evaluation for regulation is difficult. In this study, we predict the activity of every compound of DPM by using activity prediction models and select potential toxicity and mechanisms using CTD. The biological activity of individual compounds of DPM was predicted using the ToxCast-based activity prediction model obtained from the previous research. Then, pathways and diseases that may be related to the chemical were analyzed through the Comparative Toxicogenomics Database (CTD), based on all biological targets predicted to be active in one or more compounds of DPM. As a result, two diseases (cancer and urogenital diseases) and two pathways (exogenous substance metabolism and arachidonic acid metabolism) showed the highest correlation with compounds of DPM. To validate this result, in vitro assay was performed in several endpoints using human bronchial epithelial cell line (Beas-2B). Expression of Sod-1, Chk1, Chk2, XPA, Xrcc-1, KRAS-1 was altered upon exposure to the compounds of DPM, Benzo[e]pyrene and Dibenz[a,h]anthracene. Also DNA damage was found in comet assay. This study shows the possibility of predicting the category of potential toxicity of chemical, and it is expected to be helpful in evaluating chemicals efficiently.Acknowledgement: This work was supported by a grant from the Korean Ministry of Environment through 'Environmental Health R&D Program' (2021003310005).

01.05.22 Exploring the Effects of Contaminant Mixtures in an Urban Stream Using Zebrafish Assays

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Most efforts to measure contaminant concentrations and their biological effects in streams and rivers are limited to single points in time or single contaminant classes, rather than prevalent mixtures known to often be present. It is cost prohibitive to monitor the large number of anthropogenic contaminants (PFAS, excess nutrients, PPCPs, PAHs, micro and nanoplastics) found in most urban waterways or to determine the health outcomes of exposure to environmentally relevant mixtures. Improving water quality requires: a) accounting for harmful biological effects of chemicals when in mixture; b) identifying problematic contaminants and b) determining their primary sources. We are using a combination of rapid bioassays and targeted analysis of compounds indicative of individual pollutant sources (e.g. Sucralose for sewage, Glyphosate for lawns, Polybutadiene for road runoff) to identify samples of highest concern in Ellerbe Creek, NC. Samples were collected at baseflow and stormflow from each of the 36 sub-catchments of Ellerbe Creek. Each vary widely in historic and current land use, population density, socioeconomic status, and the condition of their water infrastructure. Model organisms, zebrafish, were exposed to the water samples from each subbasin and the adverse biological outcomes of the contaminant exposure were assessed (i.e., development, growth, sub-cellular effects based on novel high-throughput assays). For water samples particularly toxic to the model organisms, future chemical analysis will be conducted to fully characterize the contaminant mixture within the water samples. The results will be a first estimate of the effects of contaminant mixtures, at environmentally relevant conditions, for the highly urbanized stream in Durham, NC. This tiered approach to identifying contaminant sources of concern can speed discovery and hence water quality management in complex urban watersheds.

01.05.23 New Approach Methodologies (NAMs) and Standardized Whole Organism Models in the Context of Large Scale Chemical Monitoring

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Vital human activities such as agriculture and industrial production result in emission of synthetic chemicals (such as pesticides, industrial chemicals, or pharmaceuticals). The potential combined effects from these emissions are perceived to threat terrestrial and aquatic ecosystems. Environmental Risk Assessment (ERA) establishes a science-based framework in which both, exposure and effects are characterized to determine the likelihood (risk) of detrimental effects to non-target organisms. ERA typically follows a tiered approach in which lower tiers screen out "no concern" versus "potential concern" situations to determine if further studies are required at higher tiers (i.e., via improved and detailed exposure and or/effects characterizations) to further evaluate potential concerns identified at lower tiers. Further, component-based additivity methods such as Concentration Additional (CA) and the Maximum Cumulative Ration (MCR) allows to understand the total risk and level of complexity of exposure coming from multiple chemicals. NAMs present great promise to fasten the speed by which chemical safety screening can be performed, or to significantly expand the chemical universe that can be evaluated for risk assessment using component-based mixture assessment in combination with chemical monitoring data. However, an important element that needs detail study is the relationship and relative sensitivity of NAMs versus standardized whole organism models. This is so to understand their relationships to be able to read across them, and to be able to stablish informative levels of concern for NAMs that warrant the same level of protection as for standardized whole organism models. In this work, we explore several case studies in which existing large scale chemical monitoring data is combined with public hazard information based on both standardized whole organism models and NAMs (Tox21 data). In this project we are trying to stablish meaningful relationships between them in order to find pragmatic ways in which NAMs can be integrated in a Tiered Risk Assessment framework with various applications.

01.05.25 Toxicity Screening Models Based on ToxCast Data Using Various Machine Learning Algorithms and Molecular Fingerprints

S. Bea, J. Choi, University of Seoul / Environmental Engineering

The computing technique is growing rapidly, machine learning and deep learning approaches have been increasingly used in almost every fields. These technologies require a lot of data to use them effectively. High-throughput screening (HTS) is used to produce large amounts of data in fields such as biology, pharmacology, immunology, and toxicology. ToxCast program is one of project which using the HTS technique to screen biological activity that may suggest potential toxic effects. ToxCast program consists of more than 1400 ToxCast assays and more than 9000 chemicals. In this study, we trained the activity prediction model by using 6 algorithms (Gradient Boosting Tree, Random Forest, Multi-layered Perceptron Network, k-Nearest Neighborhood, Logistic Regression, Naive Bayes) and 5 molecular fingerprints (MACCS, Morgan, Layered, RDKit and Pattern fingerprint) alternately for each ToxCast assay. 796 ToxCast assays were selected for use in model training and every data was curated by several criteria. Generating molecular fingerprints and model training were performed in KNIME platform. The synthetic minority oversampling technique (SMOTE) was used to balance the data, and Accuracy and F1 score were used as evaluation criteria of model performance. As a result, the model with the highest performance was selected, one for each 796 assays, and among them, 34 usable models were selected based on F1 score and accuracy. The amount of data will not change for the time being due to the characteristic that HTS data is difficult for individuals to produce. Therefore, it is important that utilize and study existing HTS data. We presented an activity prediction models, which can predict various activities of substances without additional experiments using HTS data.

We expect that our result can be used as a cornerstone for a wider range of research.Acknowledgement: This work was supported by a grant from the Korean Ministry of Environment through 'Environmental Health R&D Program' (2021003310005).

01.05.26 Modernizing the Chemical Response to Oil Spills: Ecological Effects Research Forum (CROSERF) Protocol Through International Collaboration

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The Chemical Response to Oil Spills: Ecological Effects Research Forum created a standardized protocol for comparing the in vivo toxicity of physically dispersed oil to chemically dispersed oil to support science-based decision making on the use of dispersants in the early 2000s. Investigators over the years have made many modifications to the CROSERF protocol since its original inception to support the use of data generated in a more diverse manner, to incorporate advances in technology, or to test adapt to unconventional and heavier oils. Additionally, many published lab based oil toxicity studies modified the protocol without illustrating how these modifications affect the media chemistry and the resulting toxicity or omitted details necessary for inclusion in data user applications (e.g., risk assessment, modeling). The CROSERF modernization project was initiated through the Multi-Partner Research Initiative of Canada's Oceans Protection Plan to address both issues by convening working groups of international oil spill experts from academia, industry, government, and private organizations to provide the best-in-science recommendations. These recommendations will be summarized in a journal series dedicated to the CROSERF modernization efforts that includes a review of the original and revised CROSERF protocols currently used and the presentation of an adaptable and modernized protocol for future use. The finding and recommendations from the working groups in this series will provide insight into the hows and whys of oil toxicity testing to increase understanding of the comparability problem with complex media.

01.06 Contaminant Impacts on the Gastrointestinal System and New Technology Helping Advance Gastrointestinal Toxicology

01.06.01 Alkaline Phosphatase Hydrolysis of a Fluorinated Surfactant Using In Vitro Models of the Intestine and Gut Microbiome

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The polyfluoroalkyl phosphates (PAPs) are fluorinated surfactants used as grease repellents on food contact paper. The PAPs have been shown to migrate from food contact paper into food. They are found at relatively high levels in household dust, presenting sources of exposure to this class of per- and polyfluoroalkyl substances (PFAS). Correspondingly, previous research has reported PAPs in human blood from several countries. The enzyme-mediated hydrolysis of PAPs to the fluorotelomer alcohol (FTOH) has been elucidated in microbially active sewage sludge and using purified bovine alkaline phosphatase. Given that FTOHs can undergo further metabolism to bioactive and highly persistent products, it is important to understand the tissues and enzymes responsible for FTOH formation. Here, we compare the contribution of the intestine as a typical active tissue for enzyme-mediated hydrolysis to the mammalian microbiome. We incubated a representative PAP, the 8:2 monoPAP, in human and Sprague-Dawley rat intestine S9 fractions and monitored its immediate hydrolysis product, 8:2 FTOH, by GC-MS. Human and rat fecal samples were also collected, used as a surrogate for the gastrointestinal microbiome. Enzyme hydrolysis kinetics were measured and compared. Results show that the human and rat intestinal phosphatases both have 5-7 times more affinity for 8:2 monoPAP transformation (K_M (human) = 1.6 ± 0.4 µM; K_M (rat) = 1.2 ± 0.3 µM) than microbiome transformations (K_M (human) = 9.5 ± 5.2 µM; K_M (rat) = 6.4 ± 1.3 µM). These results show that while the intestine is the primary site for metabolism, we should not overlook the gut microbiome's role. The gut microbiome may impact the relative risk of PFAS exposure, given that levels of bioactive metabolites may fluctuate depending on environmental and genetic factors leading to microbial diversity across individuals.

01.06.02 Gut and Vascular Defects in Pcb-Exposed Zebrafish Embryos

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Polychlorinated biphenyls (PCBs) were used in industry for decades during the early 19th century due to their high stability under stresses. PCBs have since become a significant environmental hazard as they were discarded in waterways and soils. While having a high chemical stability, biologically PCBs are active. Small disruptions of cellular function due to PCB exposure can substantially impact overall form and function of an organ. Negatively impacting early development, PCBs cause morphological defects, induce cancer, and reduce survival in vertebrates. Long term survival of most animals is dependent on its ability to digest and absorb nutrients. The gastrointestinal system, especially the digestive tract, is functionally interconnected with the cardiovascular system in both adults and embryos. While it is clear that significant cardiovascular defects caused by PCBs exposure likely reduce the fish's lifespan, the gastrointestinal system may also be impacted by these contaminants. In zebrafish embryos exposed to individual PCBs (126 or 104) or PCB mixtures (Aroclors 1016, 1248, 1254, 1260) development is disrupted at many stages, impacting multiple organ systems. The liver, pancreas, heart, and vasculature all present with developmental abnormalities and/or have delays in maturity. Exposed embryos also show varying degrees of digestive tract abnormalities. Progression of developmental stages of the vasculature surrounding the yolk ball is delayed or halted, suggesting that not only do PCBs disrupt the ability of embryos to absorb nutrients early from the yolk ball, but coupled with the abnormalities observed in the developing gastrointestinal tract, they may have reduced ability to absorb nutrients as larvae when they start ingesting food. These defects in the gastrointestinal and cardiovascular systems are linked and potentially the root of delayed or stunted growth in exposed embryos.

01.06.03 Shifts in Riparian Spider Microbiome Communities and Declines in Endosymbionts After Elevated PCB Exposure

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Microbial communities including endosymbionts play diverse and critical roles in host organism biology. Microbial dysbiosis (an imbalance of microorganisms on or within animals) can be indicative of the host's health and potentially reflect environmental exposures to contaminants. Bacterial endosymbionts manipulate host reproduction and the loss of endosymbionts may reduce the host's reproductive success. In freshwater ecosystems, riparian predators such as tetragnathid and araneid spiders are used as biological monitors of aquatic ecosystem condition because they closely track contaminated aquatic sediments. Here, we demonstrate a significant alteration of the microbiome community including a reduction of associated endosymbionts within two riparian spiders (*Tetragnatha sp.* and Araneidae) collected from Lyons Creek East, Ontario, Canada in 2019. Lyons Creek East is an area of concern due to contamination by a transformer leak and has a gradient of PCB contamination in the sediments with some areas exceeding the screening-level environmental-quality criteria for PCBs. We extracted and sequenced the spider microbiome via the 16S rRNA gene and processed raw sequences into amplicon sequence variants (ASVs) using the DADA2 pipeline and the Silva database to determine taxonomic assignments of microbial assemblages including the endosymbiont community. Overall, araneids had higher alpha diversity of ASVs than tetragnathids but elevated PCB exposure increased this measure in araneids and some tetragnathids. Community composition of tetragnathids from the elevated PCB sites were significantly altered compared to tetragnathids collected from the low PCB contaminated sites. Interestingly, this pattern was not observed in araneids. In the low PCB sites, the endosymbiotic composition of tetragnathids was largely dominated by *Rickettsiella*, while Araneids had multiple endosymbiotic genera present, including *Wolbachia, Rickettsiella* and *Rickettsia*. Spiders collected from sites with elevated PCBs showed a sharp reduction in the presence of endosymbionts in their microbiomes. Composition of the endosymbiotic community was greatly altered, and in some riparian spiders, endosymbionts were entirely absent from spiders collected from areas with elevated PCB contamination. Overall, this has potential implications for spider reproductive success and food webs, as riparian spiders are critical gatekeepers of energy and material fluxes at the water-land interface.

01.06.06 Effect of the Gastrointestinal Environment on Desorption, Bioavailability, and Uptake of Contaminants During Dietary Exposure to Single-Walled Carbon Nanotubes

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Emerging evidence suggests that particulate contaminants, such as microplastics and nanomaterials, can accumulate other pollutants on their surface. In aquatic systems, these environmental adsorbents are present in the water column and sediments and have been identified in the gastrointestinal (GI) tract of various biota. Due to their ability to sequester toxic organic compounds, such as pesticides, flame retardants, and pharmaceuticals, environmental adsorbents may act as contaminant delivery vehicles in aquatic species. This is especially a concern during dietary exposures, where direct interactions between an adsorbent and the GI environment can potentially lead to desorption of sorbed pollutants. To assess the role of the GI system on contaminant desorption, we conducted a series of feeding experiments with largemouth bass (*Micropterus salmoides*). Bass were co-exposed via diet to single-walled carbon nanotubes (SWCNTs), a model adsorbent, and three common chemical contaminants of varying hydrophobicity: venlafaxine (VEN), ethinyl estradiol (EE2), and dichlorodiphenyldichloroethylene (DDE). Direct and indirect measurements of contaminants were utilized to assess the tissue uptake and binding affinity of SWCNTs for the chemicals of interest. Results from moderately hydrophobic contaminants (EE2 and VEN) revealed that these contaminants rapidly desorb from SWCNTs once they reach the gastrointestinal system. Results from DDE, an extremely hydrophobic contaminant, are currently being analyzed and will be presented as well. To determine which biomolecules may be driving contaminant desorption, we conducted *ex vivo* experiments with GI proteins and bile which revealed that the lipophilic components of bile are likely the primary driver of desorption. Contaminants that become adsorbed to particles and/or organic matter are typically considered the be unavailable for bioaccumulation in aquatic organisms. Our results indicate that chemicals that are absorbed to hydrophobic particulate contaminants, such as SWCNTs and microplastics, may become bioavailable during dietary exposures. Therefore, in scenarios where dietary exposure is possible, exposure to organic contaminants that are adsorbed to particles should be considered in risk assessments.

01.06.07 Exposure to Crop Production Alters Cecal Metabolome in Wild Prairie Grouse

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Although three quarters of North American birds declined during the last several decades, grassland birds experienced much steeper decline than birds inhabiting other biomes. The steep decline of grassland birds has been associated with the detrimental effects of pesticides used in modern crop production. Pesticides deplete wildlife food resources and hinder animal growth, development, and reproduction. Some authors argue that suppression of the immune system by pesticides, which facilitates emergence and spread of infectious diseases with considerably elevated mortality rates, poses the greatest, imminent threat to wildlife populations. Wildlife health and physiology are intricately connected to the gut microbiota composition and function. In our earlier study, we demonstrated that exposure to modern crop production detrimentally affects cecal microbiota of the sharp-tailed grouse (*Tympanuchus phasianellus)* and greater prairie chicken (*T. cupido*). Birds residing in the crop producing areas exhibited symptoms of dysbiosis: decline of many beneficial microbes, proliferation of pathogens, higher virulome and resistome loads and richness compared to birds inhabiting uncultivated grasslands. To elucidate how dysbiosis translates into functional physiological differences, we surveyed 928 metabolites in the cecal content of the same specimens. Over 240 metabolites were significantly over-represented in exposed birds of both host species and over 110 were underrepresented. PCA revealed that exposure to crop production had far greater effect than host species. Clusters of exposed and unexposed birds were completely separated on a PCA plot, whereas host-specific clusters overlapped moderately within exposure categories. Random forest analysis was able to classify all *T. phasianellus* to exposure category and all but a single *T. cupido*. Lipids and xenobiotics were the main drivers of exposure classification in both host species. Although some individual metabolite differences appear to be related to the dietary shift in the exposed birds, others were indicative of a perturbed microbiota composition, its altered cellular proliferation or catabolism, a leaky gut, and altered gut-liver axis in the exposed birds.

01.06.08 Gut Microbiota Alters Bioenergetics in Developing Zebrafish

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The gut microbiome can shape a host phenotype by interacting with physiological processes such as neurodevelopment, metabolism, and immune response. Emerging studies indicate the need to better understand the role of microbiota as a target of xenobiotics and as a mediator of toxic effects by altering the toxicodynamics of xenobiotic exposures. The mechanisms by which gut microbiota interact with and modify these effects remain poorly characterized. We address this knowledge gap by examining how the mitochondrial toxicities of polycyclic aromatic hydrocarbons (PAHs), ubiquitous environmental contaminants, are modified by microbiota, using zebrafish as the model organism. As a first step, we derived germ-free (GF) zebrafish and compared their bioenergetic profiles with conventionally colonized and GF zebrafish that were colonized with microbiota from adult zebrafish guts. Oxygen consumption rates (OCR) were measured using an extra cellular flux analyzer at 6 days of post fertilization (6 dpf). Lower basal metabolic rates and spare capacities (robust functional parameters of mitochondrial health) were observed for the GF zebrafish compared to the conventionally colonized cohort. Interestingly, when the microbiome was introduced back to the germ-free zebrafish, the ramping up of these parameters was observed. These results along with the NADH production data provide strong evidence that the gut microbiome plays a crucial role in mitochondrial function and bioenergetics. Based on these preliminary data, research is under way to determine the changes in mitochondrial function in PAH-exposed fish when colonized with different microbial communities, including that of the microbiomes of fish inhabiting PAH contaminated habitats. Collectively, these studies will provide information on the role of the gut microbiome in mediating xenobiotic toxicity in an ecological context.

01.06.09 Transcriptomic Points of Departure for Human Intestinal Cells Exposed to Dietary Nanoparticles

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Engineered nanoparticles (NPs) are increasingly being used in the food sector, yet little is known about the potential health risks associated with their dietary exposure. In this study, we investigated the most widely used NPs in food industry, including food grade silicon dioxide (SiO_2), titanium dioxide (TiO_2) and silver (Ag), along with their non-food grade and bulk counterparts. The concentration-dependent effects were studied by exposing 1-100 ppm of particles to two human intestinal epithelial cell models (Caco-2 and HIEC-6) for 24 hours followed by characterizing a reduced representation of the cell transcriptome (i.e., L1000 genes) using targeted RNA sequencing. Differential expression of genes was calculated based on cells exposed to serum free culture media. Preliminary data analysis identified elevated expression of marker genes in the oxidative stress, cellular junction complex, and pro-inflammatory signaling pathways. Transcriptomic points of departure were calculated, with most potent concentrations found for AgNPs, and in particular with respect to apoptotic responses and cancer pathways. The points of departure concentrations calculated for SiO_2-exposed cells are relevant to human exposure conditions, suggesting the possibility of adverse effects due to daily exposure. The findings from this study suggest that chronic exposure to dietary NPs may underlie the development and progression of gastrointestinal disorders.

01.06.10 Into the Abyss: Summarizing the Past and Identifying Key Future Directions of the Intestine in Aquatic Toxicology

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It is acknowledged that the intestine is important from its role in general health, growth ,reproduction and immune function, with the intestinal barrier playing a key role in protecting organisms from deleterious affects associated with bacteria and various chemicals. The gut microbiome is increasingly recognized as a key part of host xenobiotic biotransformation through direct and indirect mechanisms. In the aquatic environment, an oral route of exposure is perhaps the most relevant when linking gut microbiome perturbations and chemicals because aquatic organisms are exposed to environmental chemicals through the water and food. Water-soluble chemicals or those adhered to food particles can be ingested, where they can interact with gut epithelial receptors before or after microbial transformation. A specific example includes the ingestion of PAHs bound to food, which activates AhR in the gastrointestinal tract. However, less is known about the affect and impact of the intestine on metabolism in general. In this study, we examine the available literature to identify key gaps in the literature. Types of studies, and methods typically employed will be summarised and compared to the mammalian literature in order to identify potential new methods to employ to enhance understanding the contribution of the intestine to chemical metabolism. Logical steps to expand and target gaps in the knowledge to directly enhance knowledge of extrahepatic metabolism focusing specifically on fish will be outlined.

01.07 Environmental Fate and Effects of Nanomaterials

01.07.01 Copper Oxide Nanoparticles' Effects on the Growth and Development of Rice and Mechanism of Inhibiting Arsenic Accumulation

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Rice and its products are the main chronic exposure route of arsenic (As) to humans via diet. Conventional approaches to mitigate As pollution and inhibit As accumulation in rice plants, e.g., physical turnover, chemical remediation, have low efficiency or are not cost-effective, etc. Application of copper oxide nanoparticles (nCuO) is promising in emerging nano-agriculture, because nCuO can alleviate As phytotoxicity and inhibit As accumulation in rice grains. However, the dominant influencing factors on nCuO environmental behaviors are dependent on specific paddy conditions, which needs to be further studied. The mechanisms of the interaction between nanoparticles (NPs) and As in the environment or within the plants are still unclear. This project intends to simulate rice plant growth in a greenhouse with certain As concentrations in the soil collected from southern China where paddies were seriously polluted by As, and introduce nCuO by either adding into the water phase of the growth media or spraying onto the leaves of rice plants at different growth stages of rice. The concentrations of nCuO used were derived from our previous studies, where we got the conclusion that nCuO at 50 mg/L increased rice yield and inhibited As accumulation. In the previous studies, we also explored the basic behavior of nCuO and As in hydroponic solutions and simulated paddies to investigate their interaction in the environment. In this project, by using techniques such as inductively coupled plasma mass spectrometry, transmission/scanning electron microscopy-energy dispersive spectrometer and other techniques to: 1) explore the environmental behaviors of nCuO and As and their influencing factors in the environment, e.g., natural organic matter and phosphate; 2) to investigate the mechanisms (e.g., adsorption, redox and microbial activity changes) of the co-transport of nCuO and As, and the interaction between nCuO and As within rice plants (e.g., inhibiting the activity of As transporters). The results would provide a theoretical basis for the practical application of NPs in agriculture, such as maintaining the efficiency of nCuO and applying nCuO with the optimal method and at the optimal rice growth stage.

01.07.02 Transgenerational Changes in Arabidopsis tsRNA Expression and Chloroplast Genomic Methylation Following Exposure to CeO2 Nanoparticles

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Crops and wild plants are being exposed to increasing amounts of engineered cerium oxide nanoparticles (CeO_2-NPs) through soil, water, and air. Recent studies showed that exposure to CeO_2-NPs can alter the transcriptome profiles of plants. To further identify transgenerational molecular impacts from exposure, experimental groups of *Arabidopsis thaliana* plants were grown for three generations. Treatments with 15 mL of 500 mg/L CeO_2-NP +/- treatments occurred in the 1st and 2nd generations (C1C2 = control both generations; T1C2 = treated 1st generation; C1T2 = treated 2nd generation; T1T2 = treated both generations). No treatments were applied during the 3rd generation. Leaves from 28-day-old 2nd and 3rd generation plants were sampled (N = 5) for small RNA sequencing and reduced representation bisulfite sequencing, respectively. Differentially expressed transcripts were predominantly tRNA-derived small RNAs (tsRNA) that were cleaved within the anti-codon loop, and which are capable of regulating translation. For T1C2 plants there was significant up-regulation of 5′ tRH-Asp-GTCs, whereas C1T2 had up-regulation of 5′ tRH-Gly-GCCs. Interestingly, for T1T2 plants there was a switch to down-regulation of 5′ tRH-Gly-GCCs. We used 124,208 100-CpG probes to quantitate differential methylation at genes across the genomes of 3rd generation plants. Notably, 84% of the genes with differentially methylated CpGs were in the chloroplast genome, while only 3% of all 100-CpG probes were generated there. The most wide-spread enrichments of gene ontologies across treatments were those related to ATP synthesis, electron transport and photosystem II. The most frequently impacted biochemical pathway was also ATP synthesis. Overall, these results point to development of epigenetic memory of particular CeO_2-NP exposures that occurred in prior generations. The tsRNA expression was more sensitive to exposures in just the 1st or 2nd generations (T1C2 and C1T2). By contrast, 2nd generation exposures (C1T2 and T1T2) had the largest effect on genomic DNA methylation of 3rd generation plants. Results from these experiments suggest that CeO_2 exposure during the 1st generation attenuated the responses to a follow-on exposure in the 2nd generation.

01.07.03 Impact of TiO2-NPs in Wastewater on Heavy Metal Uptake by Potato Plants

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Increasingly considered as a source of water for irrigation, wastewater (WW) can, however, pose health risks due to contaminant uptake by crops. Contaminants in WW, including heavy metals and organic contaminants, are known to be harmful to the environment and human health. The detection of nanoparticles (NPs) in WW is raising questions about their potential interactions with co-contaminants and their effects on soil-water-plant systems. Titanium dioxide NPs (TiO_2-NPs), a type of nanoparticles commonly used in many industries and reported to be present in wastewaters can potentially affect the soil mobility of contaminants and crop uptake of heavy metals. A field lysimeter study was carried out for two years (2017 and 2018) to investigate the impacts of TiO_2-NPs on the mobility of heavy metals (Cd, Cr, Cu, Fe, Pb and Zn) and their uptake by potato plants. Potatoes were grown in sandy soil under controlled conditions and irrigated with synthesized WW or WW + TiO_2-NPs. At harvest, the heavy metal concentrations of potato tubers and plant parts were determined using inductively coupled plasma mass spectrometry (ICP-MS). The presence of 1000 ug/g of TiO2-NPs L^{-1} under WW irrigation water significantly (P £ 0.05) reduced uptake of Cd, Cu, and Zn into potato flesh, skin, and roots in both years, but had no significant effect on Cr, Pb and, Fe uptake into any plant parts.

01.07.06 The Effects of Complex Aqueous Matrices and Exposure Route on the Bioaccumulation Dynamics of Nanoparticle Silver in Daphnia magna

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Treatment wetlands for water purification are typically designed to allow for various physical and biological processes to reduce levels of organic contaminants, metals, bacteria, and suspended solids. Grazing by filter-feeding zooplankton such as Daphnia magna can remove a variety of pollutants in treatment wetlands. Silver nanoparticles (AgNP) are widely used due to their antimicrobial properties, so the environmental concentrations of AgNP and dissolved Ag are likely to increase. Silver is toxic to aquatic organisms, but the effects of AgNP on zooplankton such as D. magna are not fully understood. Daphnid exposure to AgNP may result in negative health effects including a reduction in filter-feeding abilities. Understanding the impacts of AgNP on zooplankton is critical to predict treatment wetland function and mitigate the risks of discharging inadequately treated water. Our overarching research objective is to characterize and compare aqueous and dietborne uptake and loss of isotopically labeled citrate-coated 109AgNPs and 109AgNO3 in D. magna. Using isotopically labeled silver enabled experimentation at environmentally relevant concentrations (i.e. 0.01 - 2.5 µg L-1 Ag). We show that both forms of Ag are bioavailable and that there is greater retention of AgNP compared to AgNO3, which may indicate the potential for bioaccumulation and biomagnification at different trophic levels. Both waterborne and dietborne uptake result in measurable influx and retention of Ag. Evidence of feeding inhibition through dietary exposure at higher concentrations of Ag may have negative impacts on daphnid growth and reproduction, which could reduce treatment wetland efficacy. We also examined the effects of water chemistry on Ag influx, showing the importance of using environmentally relevant media when considering environmental fate and toxicity. The findings from these studies provide environmentally relevant data that helps elucidate ecological and environmental risk associated input of AgNPs in natural treatment systems.

01.07.07 Silver and Titanium Dioxide Nanoparticles' Mechanism of Toxicity in the Fish Intestinal Cell RTgutGC

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Titanium dioxide (TiO_2-NP) and silver nanoparticles (AgNP) are used in several consumer products and represent a large proportion of all nanoparticles produced every year. This raises credible questions about their impact on human health and the environment. Due to their distinct physicochemical properties (TiO_2-NP do not dissolve while AgNP dissolve in solution) they represent an interesting model to study nanoparticle mechanism of toxicity. The fish intestine is an important route of uptake for metals and metal nanoparticles. In this study, we used an intestinal cell line from rainbow trout (*Oncorhynchus mykiss*), the RTgutGC, which when cultured on permeable membranes forms a polarized epithelium that closely mimics the physiology of the gut epithelium *in vivo*. We used the RTgutGC model system to determine intracellular chemical transformations by X-ray absorption spectroscopy and transcriptional responses by RNA-Seq in cells exposed to silver nitrate, AgNP and TiO_2-NP for 1 to 142-hours. We also mapped the intracellular metal concentrations by X-ray florescence microscopy. Our data showed that while silver and AgNP induced toxicity and rapid transcriptional and morphological changes, TiO_2-NPs did not. Upon entry, dissolved silver was rapidly complexed with cysteine and was translocated inside the cell nucleus. Silver nanoparticles uptake was slower but over 142 hours about 30% of total silver dissolved from the nanoparticle surface forming cysteine complex (~20%) and free ionic silver (~10%). The transcriptomic analysis showed a clear segregation of all treatments and time points. Moreover, gene enrichment analysis showed that apoptosis was among the most induced pathways by silver and silver nanoparticles which was evidenced also by morphological changes such as nuclear condensation. This study presents a comprehensive investigation of silver and titanium nanoparticle toxicity and shows that ionic dissolution is a major driver of acute toxicity.

01.07.08 Optimization and Validation of High pH Tissue Extraction Methodologies for 2D Nanomaterials in Biological Matrices

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The use of engineered nanomaterials (ENMs) in a variety of consumer and industrial products has raised concerns regarding environmental and human health risks during material life-cycles. The environmental fate and toxicity of the growing class of two-dimensional (2D) ENMs is especially limited. Some 2D materials interact with biological receptors directly. Interactions may also occur as a result of exposure to 2D materials that subsequently undergo transformations, and these interactions are unique to the ENM exposure. Analytical methods to extract and quantify ENMs have not been validated for 2D materials. ENM analysis often employs tetramethylammonium hydroxide (TMAH) extraction followed by single particle inductively coupled plasma mass spectrometry (spICP-MS). Although effective, tetramethylammonium is a known neurotoxin, limiting use of TMAH in many laboratories. The objectives of this research were to evaluate alternatives to TMAH extraction, and evaluate the applicability and limitations alkaline digestion for 2D ENMs using 2D MnO_2. Tetrabutylammonium hydroxide (TBAH) and tetrapropylammonium hydroxide (TPAH) were evaluated as alternatives to TMAH due to structural similarities and lack of similar, reported toxicological effects. In addition to 2D MnO_2, controls using 60 nm Ag and Au ENM allowed comparison to published TMAH procedures. Reactors containing each ENM individually were made using 20% solutions of TMAH, TBAH, and TPAH with and without a model mammalian tissue (ground beef, 96% lean). Reactors were sampled at regular time-intervals starting at 24 hours and continuing for 6 months to evaluate sample holding time. Analysis at each time point was split to determine the nanomaterial and dissolved elemental fractions. ENM analysis was conducted using spICP-MS, and subsamples were centrifuged and analyzed using traditional ICP-MS to determine dissolved elemental concentrations. ENM recovery varied between bases and ENMs, but generally, recoveries are lower than 75% after 7 days for all ENMs and all bases in tissue-free reactors. Particle mass distributions did not change, suggesting loss is attributable to reactor walls rather than dissolution, although analysis is ongoing. Analysis of ENMs from biological tissue (ongoing) is expected to yield higher recovery, consistent with prior studies of Au and Ag ENMs using TMAH. Thus, this improved method will be an important tool for understanding any risks associated with 2D ENMs.

01.07.11 Effect of Iron Oxide, Copper Oxide and Silver Nanoparticles on the Growth of Kidney Bean Seedlings and Soil Microbial Community

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The increase in the development and application of nanoparticles (NPs) has made their dispersal into the environment inevitable, thus raising concerns on the potential risk to agroecosystems. It is crucial to understand the impact of NPs on the growth of food crops and soil microbial community. In this study, we examined the impact of polyvinylpyrrolidone (PVP)-coated and non-coated Fe_3O_4, Cu_2O and Ag_2O NPs on soil microbial community and on early growth stages of kidney bean plants. Kidney bean seeds were soaked in NP solution and placed on a shaker for 4 hrs. Seeds were soaked in 20, 100, 500 mg NPs/40ml of nanopure H_2O and 0 mg NPs/40ml of nanopure H_2O (control treatment). Afterwards, the seeds were grown in a potted soil system for 30 days under a controlled environment. The percentage of seed germination in the control treatment was higher than in the Fe_3O_4, Cu_2O and Ag_2O NPs treatment samples. The germination rate decreased with an increase in the concentration of Fe_3O_4 NPs while Cu_2O and Ag_2O NP treatments had no effect on germination rate at the concentrations tested. The Cu_2O and Fe_3O_4 NP treatments increased the shoot growth while soaking seeds in 12.5mg/ml of Ag_2O resulted in reduced growth of the germinated seedlings. The inhibition of seedling growth by Ag_2O is possibly attributable to bioaccumulation of elemental Ag in the plant tissues. There was no difference on the effect of the PVP-coated and non-coated NPs treatments on the root and shoot growth of the kidney bean plant. In addition, we explored the effect of Fe_3O_4, Cu_2O and Ag_2O NPs on soil microbial community. Soils were thoroughly mixed with 500 mg of Fe_3O_4, Cu_2O, Ag_2O NPs and control (0 mg) per kg of soil and incubated for 30 days. Soil samples were collected 24 hours after NP addition (day 1) and at 30 days. Using real-time polymerase chain reaction (RT-PCR), we investigated how NPs influence the abundance of some commonly occurring taxa of soil bacteria and fungi including *Alphaproteobacteria, Betaproteobacteria, Firmicutes, Actinobacteria* and *Bacteriodetes*. The *Actinobacteria* and *Bacteriodetes* were lower in abundance than other groups of bacteria - *Alphaproteobacteria, Betaproteobacteria* and *Firmicutes* 24 hours after the addition of Fe3O4, Cu2O and Ag2O NPs while Actinobacteria further decreased in abundance at day 30. We are still exploring the effect of Fe_3O_4, Cu_2O and Ag_2O NP treatments on the abundance and composition of soil microbial community using 16S rRNA sequencing method. While the studies to determine the long-term effects of NPs on soil microbial community and abundance are ongoing, these initial results suggest that NPs exposure to the environment will likely pose potential risks to the environment and the mechanism of their effects on organisms require careful analysis.

01.07.12 Soil-Exposure Effects of Lithium Cobalt Oxide (LCO) Nanoparticles to Immature Soybean (Glycine max)

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Portable electronics rely on advanced battery technology that incorporates the use of lithium-ion lithium cobalt oxide (LCO). These batteries are crucial for powering devices for long periods of time, nevertheless, they have short lifespans and are not recycled. Due to this, it is vital to evaluate their impact on the environment after disposal. Exposure of LCO nanoparticles to invertebrates has altered Fe-containing protein function, reducing hemoglobin production. Soybean and other leguminous plants require oxygen-carrying proteins like leghemoglobin to facilitate nitrogen fixation. Currently, the effects of LCO nanoparticles on leghemoglobin production or function are unknown. To assess the effects of these nanoparticles to leguminous crops, soybeans were grown in soil amended with LCO nanoparticles at concentrations of 0, 10, 25, 50, and 100 mg L^{-1} alongside equivalent amounts of Li and Co ions. After 28 days of exposure, fresh biomass, total chlorophyll, total protein, nodule mass, and enzyme activity related to oxidative stress were evaluated. Additionally, elemental analysis was performed to measure the uptake of Li, Co, and macro/micronutrients in root, aerial, and nodule tissues.

01.07.13 Developmental Hazard Associated With Zebrafish Exposures to Some Nano and Micro Graphene Oxide Materials

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Graphene and its derivatives, as two-dimensional nano and micro sheets, have desirable physicochemical properties leading to an ever-expanding list of industrial and biomedical uses. One subcategory of the class is graphene oxides (GOs). While much has been learned about their human safety, their potential for developmental hazard is still largely unknown and the number of new GOs is outpacing our efforts to understand their impacts on biological systems. Our previous work advanced the use of zebrafish to interrogate the interactions between engineered nanomaterials and biological systems. Here we sought to interrogate this for GO 250x250 nm, 400x400 nm, 1x1 µm; partially reduced GO (prGO) 400x400 nm; reduced GO (rGO) 400x400 nm, and 2x2 µm. These GOs first underwent extensive materials characterization under support from the NHIR Consortium, of which our laboratory is a participant. prGO and the rGOs were stably dispersed in sodium cholate, whereas the other GOs were stabilized in water (GOs in water (GOW)). Zebrafish embryos were exposed to 0, 2.32, 5, 10.7, 23.2, and 50 µg/mL starting at 6 hours post fertilization (hpf) until 120 hpf. Embryos tolerated cholate normalized to 400 µg/mL without effect. Exposures to 400x400 nm GOW (mGOW), 1x1 µm GOW (lGOW), prGO 400x400 nm, and rGO 2x2 µm were associated with significant teratogenicity. When sodium cholate stabilization was added to the GOWs to test if cholate altered the toxicity, we observed a significant increase in toxicity compared to the regular GOW. mGOW was found to be the most toxic material in the study causing significant mortality within 1 h of exposure commencement. Using mGOW at 50 µg/mL as the representative of GO hazard, we determined that the toxic effect manifested in the yolk syncytial layer (YSL). Using brightfield imaging and scanning electron microscopy, we were able to observe contents leaking from the yolk sac and instances of accumulation of mGOW on the animal pole. These effects may be particularly relevant to biomedical applications of GOs in human health.

01.07.14 Framework for the Risk Assessment of Manufactured Nanomaterials Under the Canadian Environmental Protection Act, 1999

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The Government of Canada is developing a nanomaterial regulatory risk assessment framework to inform the assessment of manufactured nanomaterials under the *Canadian Environmental Protection Act, 1999* (CEPA), including existing nanomaterials in commerce in Canada, and new nanomaterials notified prior to being manufactured or imported into Canada. The development of this framework is built upon successive efforts, arising from work begun with the Canada-US Regulatory Cooperation Council on nanotechnology (2011-2014), and evolving through consultations conducted on the approach to assessing nanoscale forms of substances on the Domestic Substances List (DSL) (2015-2018). The framework leverages the general practices used for the assessment of bulk substances in Canada, and outlines approaches and key considerations on the assessment of environment and human health risk related to nanomaterials (e.g., unique properties of nanomaterials). Specifically, these approaches and key considerations include grouping strategies, physical-chemical considerations, environmental fate and persistence, data considerations, approaches to characterize the exposure and effects, and developing the risk characterization of nanomaterials to determine if a nanomaterial meets Part 5, Section 64 of CEPA. The Framework has been developed to provide guidance to regulators on how to assess nanomaterials, while its publication serves to inform stakeholders and the general public about the approaches and considerations used by the Government of Canada for assessing NMs under CEPA. Canada will be the first regulatory jurisdiction to publish a risk assessment framework specific to nanomaterials that broadly covers industrial and consumer uses. The framework has been subject to public comment, providing an opportunity for a broad engagement with all possible stakeholders and the public, as well as ensuring transparency to the risk assessment process.

01.07.15 Investigating the Impacts of Nanomaterial Chemistry and Weathering on Polymer Additive Release From Epoxy Composites

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Nanomaterials (NMs) have unique properties that differ from their bulk counterparts and can be added to polymers to create polymer nanocomposites (PNCs) with desirable mechanical, thermal, and electrical properties. Despite the benefits of NMs in PNCs, there are significant concerns regarding their potential environmental release and toxicity. These PNCs may also contain potentially toxic chemical additives and monomers such as nonylphenol, tert-butylphenol, and bisphenol A, that upon weathering can leach out into the environment. Taken together, the interactions between NMs and polymer additives within PNCs require a better understanding of the role nanomaterial chemistry plays in additive behavior. In this project, NMs (titanium dioxide, graphene oxide, and carbon nanotubes) are embedded into an epoxy resin to create a series of PNCs. In addition, a subset of PNCs also contains a common ultraviolet absorber to explore the role of photochemistry. These PNCs are weathered through field and simulated exposure and then leached in environmentally relevant aqueous media for five days, with the leachate analyzed by liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS). The PNCs were further characterized by Raman microscopy and Fourier-transform infrared (FTIR) spectroscopy. It is anticipated that nanomaterial chemistry will play a key role in additive release and transformation in the environment.

01.08 Fate and and Effects of Nano- and Microplastics in the Environment

01.08.01 Using Environmental RNA to Evaluate the Impacts of Nanoplastic on Benthic Invertebrate Communities

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Plastic particles are ubiquitous in marine systems and the effects of nanoplastic particles on marine organisms are of growing concern. Nanoplastics enter marine systems primarily through the fragmentation of larger plastics present in the environment, often ultimately accumulating in sediments. Marine sediments act as a sink for many contaminants and are rich habitats for benthic micro- and meiofauna which form the base of the marine food web. However, little is known about the sensitivities of specific species to nanoplastics or the effects on community diversity. Utilizing molecular methods, such as metabarcoding of environmental DNA/RNA, allows for rapid and comprehensive detection of microscopic organisms via high-throughput sequencing and the ability to assess community diversity and structure. The objective of this study was to use an RNA metabarcoding approach to investigate the effects of nanoplastic particles on benthic micro- and meiofaunal community diversity. Sediment cores (mesocosms) were collected from the Narrow River estuary in Rhode Island (USA) and exposed to 200 nm polystyrene beads at concentrations of 0, 0.1, 1, 10, or 100 mg/kg dry weight in sediment for two weeks. Following exposure, RNA and DNA were co-extracted from the top 1 cm sediment layer, RNA was reverse-transcribed, 18S and CO1 markers were PCR-amplified, and amplicons were sequenced on an Illumina MiSeq. Key differences in the value of environmental DNA compared to environmental RNA for ecotoxicological applications were identified. Using the 18S marker, significant differences to α-diversity and β-diversity were observed in 200 nm nanoplastic exposures relative to the control. Observed community-level differences were driven by the differential abundance of several species of protozoa. This study highlights the utility of using community endpoints to assess nanomaterial impacts.

01.08.02 Influence of Microplastics on the Toxicity of Chlorpyrifos and Mercury on the Marine Microalgae Rhodomonas lens

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The growing use of plastics, including microplastics (MPs), in commercial products has enhanced their potential release into aquatic environments, where microalgae represent the basis of food webs. Due to the physicochemical properties of MPs, they may act as carriers of both organic and inorganic pollutants. Due to the high concern and limited knowledge on the effects of MPs and associated pollutants, on primary producers, the purpose of the present study was to determine the toxicity of MPs and the model pollutants chlorpyrifos (CPF) and mercury (Hg) on the red microalgae *Rhodomona lens*. This will lead to better knowledge on the impact of MPs on marine microalgae and their role as vectors of potentially harmful pollutants. *R. lens* cultures were exposed in 96 h bioassays to 1, 10, 100, and 1000 μg/L (corresponding to 25, 248, 2475, 24750 particles/mL) of polyethylene particles with a mean size of 7.73 μm (plain MPs) and 10-15 μm (oxidized MPs), alone or in combination with CPF and Hg. The individual toxicity of CPF and Hg was also studied. The evaluated parameters were: average specific growth rate (ASGR, day^{-1}), and cellular viability and pigment concentration (chlorophyll a, c2 and carotenoids) at 48 and 96 h. No significant effects were observed in the growth pattern after 96 h exposure to plain and oxidized MPs. However, a significant increase in cell concentration was observed only at 48 h of a single exposure to plain MPs. A concentration-dependent decrease of the ASGR was observed after exposure to CPF/Hg and to CPF/Hg-loaded MPs, whereas viability was affected by the exposure to both MPs and CPF/Hg, alone and in combination. In the case of pigment content, chlorophyll a and c2 significantly decreased when exposed to plain MPs and CPF, however, both pigments significantly increased when exposed to CPF-loaded MPs. Similarly, chlorophyll and carotenoid content significantly decreased after exposure to Hg; however, a significant increase in chlorophyll a was observed after 48 h exposure to Hg-loaded MPs, at the higher tested concentration.

01.08.04 The Influence of Weathering on the Toxicity of Leachate From Ten Microplastic Types

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Chemicals released from micro and nano-plastics can be a cause of toxicity to marine organisms. The chemicals are expected to occur as mixtures and will change depending on their disposition in the environment. When plastics first enter the environment, they may release unreacted monomers from the production of the plastics or chemicals added during the manufacture. These plastics may also accumulate new chemicals from land- and marine-based sources, which can then be released as environmental conditions change. We expect that older, more weathered, plastics will have lost additives and plasticizers but would accumulate other contaminants from the sediments or water column. The influence of weathering on toxicity has been understudied but our work consistently shows that toxicity decreases when tire wear particles, nurdles, microfibers, and ground consumer products were deployed in a marine environment for 2.5 months. We generated microplastics from premanufacture polystyrene, PET, and polycarbonate; consumer polystyrene, PET (fragments), green and white polyester, and acrylic (fibers); and new and used tires of the same brand and model. A portion of each microplastic type was retained in the laboratory (non-deployed), while the remaining portion was deployed in a marine environment during peak stormwater runoff months. 96-hour LC50s were determined with < 48-hour *Americamysis bahia* in leachate generated from the microplastics. The only microplastic leachates that resulted in toxicity were the three non-deployed fibers and the new and used tires. The non-deployed tires were the most toxic followed by deployed tires, non-deployed red acrylic, non-deployed green polyester, and non-deployed white polyester. Work is ongoing to determine the chemical differences between deployed and non-deployed plastics, but the work highlights how important weathering is to consider when evaluating chemical toxicity from plastics.

01.08.06 Neurodevelopmental and Epigenetic Toxicity of Polystyrene Microplastics in Zebrafish Embryo and Human STEM Cells

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In the previous studies, it was reported that exposure of a small size of plastic particles affects (directly or indirectly) the brain and the behavior change of the model organism. In this study, zebrafish embryo and human stem cells (embryonic stem cell, neural stem cell) were used to study the neurodevelopmental toxicity of plastic exposure at an early stage of life and whether it causes epigenetic toxicity. Three sized polystyrene microplastics (PSMPs, 0.1, 1, and 10 μm) were exposed to zebrafish embryos (6hpf) for 5 days and to human embryonic and neural stem cell for 14 days. The neurodevelopmental toxic responses were investigated using larval behavior and behavior-related gene expression for zebrafish, whereas, using cytotoxicity and neurodevelopment marker gene expression for stem cells. In addition, we observed the developmental effect in the early stage of development using EB formation. At the end of the PSMPs exposure, DNA methylation, as the epigenetic markers, was analyzed to identify role of epigenetic change in neurodevelopmental toxicity. There was no significant effect on the behavior of zebrafish larva (5dpf) to PSMPs exposure, whereas significant expression of neurodevelopmental marker genes was observed. In the 14-day exposure to stem cells, a difference in toxicity was investigated depending on the size of PSMPs. Taken together, PSMPs exposure caused neurodevelopmental toxic potential to zebrafish and human stem cells. The results on DNA methylation also suggested possibility of epigenetic toxicity of PSMPs. In future studies, we plan to analyze the methylation of specific target genes to better understand the role of DNA in neurodevelopment toxicity of PSMPs. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT, Ministry of Science and ICT) (NRF-2020R1A2C3006838).

01.08.07 Consumption of Real Microplastics by Apple Snails (Pomacea paludosa): Influence of Time, Age, and Plastic Types

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The recent increase in use of plastic products, coupled with mismanagement of waste has resulted in an alarming spike in plastic pollution, with aquatic systems often becoming major sites of plastic accumulation. The presence of plastic particles in the environment poses potential threats to aquatic life, the sum of whose effects still remains unknown. This study posits three questions: 1) How does the age of the organism affect consumption? 2) How does exposure time affect consumption? 3) Are there differences in consumption according to plastic-type? The study used four plastic types: nylon (PA66), polymethyl methacrylate (PMM), polyethylene terephthalate (PET), and polylactic acid (PLA). Snails of three different ages (3 days, 2 weeks, and 1 month old) were used in this study to quantify the differences in microplastic consumption by the organism's size and maturity. Organisms of each age were exposed to one of the four different plastic types over 96 hours at 25 °C at a plastic concentration of 1 mg/L. Five replicate snails were collected at 1, 3, 6, 12, 24, 48, 72, and 96 hours post-exposure to determine microplastic consumption. Results show that snails consumed microplastics after one hour of exposure. Overall, microplastic consumption appeared to follow a pattern with exposure time and was dependent on the organism's age. The greatest consumption was observed after 96 hours of exposure. Among the plastic types, PMM appeared to have the greatest consumption at concentrations up to 329 particles/ snail after 96 hours of exposure. These findings may also provide insights as to how snail mastication and digestion may increase total particle numbers as the plastics degrade within the organisms.

01.08.08 Effects of Microplastics on Aquatic Community in Freshwater Microcosm

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Microplastics are emerging as a profound concern for the scientific community and the general public given their abilities to serve as a substrate for growth of microorganisms, to sorb organic pollutants which easily leach into organisms after ingestion, and to travel through and between ecosystems proliferating the entire planet. Research has indicated that microplastics affect living organisms including zooplankton, fish, and several others but has not yet determined their effect on freshwater phytoplankton. This study aims to fill this knowledge gap since phytoplankton are the foundation of the aquatic food web and invaluable primary producers. Specifically, this study investigates the influence of algal colonization atop the surfaces of microplastics in freshwater microcosms on the following: microplastic deposition rate, phytoplankton community structure, primary productivity, and nutrient availability. Sediment and water were collected from a local pond and added to 9 90-gallon aquaria at Loyola University Chicago to initiate the microcosm systems. Once the systems stabilized and established their baseline (around 3 months), microplastics were added in low concentration—for an environmentally relevant comparison—and in high concentration to determine the effects of plastic particles on fully established phytoplankton communities. The addition of plastics initiated the treatment phase. Three aquaria with no addition of microplastics were used as control microcosms for comparison. Over the course of the treatment phase, monitoring of microplastic deposition, phytoplankton colonization, primary productivity, chlorophyll a absorbance, nutrient concentrations, phytoplankton community structure and density, as well as water quality characteristics were conducted. Repeated-measures ANOVA for change in criteria over time and treatments will be used to analyze the significance of any change in the analyzed criteria that takes place after microplastics are added. Non-metric multidimensional scaling will be used to examine changes in phytoplankton community structure. Detailed results will be presented at the conference in November.

01.08.09 Micro-Fate - Characterizing the Fate and Effects of Microplastic Particles Between Hotspots and Remote Regions in the Pacific Ocean

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Despite high research activity regarding the occurrence, fate and effects of plastic debris in marine environments, field data is scarce, in particular covering the subsurface layers that in many cases extend to several thousand meters of depth. This presentation will give an overview of the research outcomes of our interdisciplinary project MICRO-FATE that aimed at characterizing the fate and effects of field-aged plastic particles (macro, micro, nano) in hotspots and other regions of the North Pacific Ocean. Specifically, we addressed the (i) collection, identification and characterization of plastic particles, (ii) their weathering, fragmentation and aggregation, (iii) sorption and leaching of chemicals to/from the plastic material, and (iv) biofilm growth on the particle surface, providing contributions to the current understanding of the transport, fate and potential effects of plastic debris in the marine environment.We present the major outcomes of MICRO-FATE, with a focus on the topics above: (i) Plastic particles have been collected at the surface using a neuston catamaran and with continuous sampling from a cascade filtration unit, from the water column with in-situ pumps and from the seabed using a multiple corer to collect sediments. Our data supports the expected elevated concentrations in the Great Pacific Garbage Patch. (ii) In addition to field-weathered material, we also performed on-board weathering experiments with pristine and pre-weathered polymer samples in mesocosms under flow-through conditions with local seawater, either exposed to the solar radiation or covered as dark controls. Our data assessment indicates that weathering effects were detectable already during the 4-week exposure period. (iii) For the assessment of organic pollutants, we collected floating macrolitter and surface sediment and enriched pollutants from large volumes of water (60-100 L) from the surface and the water column on solid-phase extraction sorbent which allowed for dozens of chemicals to be detected. Bioanalytical assessment of the mixtures of chemicals complements this data set. (iv) Biofilms from the surface of floating plastic debris were collected, submitted to functional analyses, characterized using confocal laser scanning microscopy and cultivated to investigate the "plastisphere" which has allowed for the identification of several organisms. Furthermore, the early colonization stages from the mesocosm experiment have been investigated.

01.08.10 Seasonal Variation of Microplastics in the Surface Waters of the River Netravathi, India

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Microplastic pollution is a global environmental problem receiving ever-increasing levels of public attention. Their negative impact on the ecosystem and life, ranging from tiny microorganisms to higher organisms, including humans, has been reported. The rivers can act as a temporary sink for microplastics, and they are the significant carrier of these materials from land to the oceans. Other than anthropogenic influences, there are non-anthropogenic factors, including rainfall which influences the distribution of microplastics. The present work is carried out to understand the seasonal variation of microplastics in the River Netravathi, a west-flowing river debouching into the Arabian Sea. Surface water samples (120 litres) were collected from the river during post-monsoon (January 2020; n = 17) and monsoon (July 2020; n = 14). Maximum recovery of microplastics from the sample was possible by following a modified version of the NOAA protocol. The mean (± standard deviation) abundance of microplastics in the surface water of the river during post-monsoon and monsoon is 15.06 (± 15.46) and 36.86 (± 23.12) pieces/ m^3, respectively. The maximum concentration of microplastics obtained is 56 pieces/m^3 (post-monsoon) and 72 pieces/ m^3 (monsoon). Four sampling sites in post-monsoon and one in monsoon showed no microplastics. Fibres predominated the samples during both seasons. Equal proportion of smaller (0.3 - 1 mm) and larger (1 -5 mm) size fractions were observed during post-monsoon, whereas larger microplastics dominated during monsoon. The relatively higher number of microplastics during the monsoon season can be due to the associated increases in the rate of surface runoff, which will carry plastic materials from the adjacent areas to the river. Following the end of the rainfall event, most of the plastic materials might be moved to the sea (flushing) or deposited in the sediments on the decrease of flow velocity, resulting in lower microplastic abundance during post-monsoon. A clear spatial and temporal variation pattern is absent, mainly due to the study area's hydrodynamic conditions and anthropogenic interventions.

01.08.11 Microplastic Sedimentation Rate and Characteristics in a Semi-Enclosed Bay of South Korea

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Microplastics are ubiquitous in the marine environment. Recent studies demonstrated that widespread occurrence of microplastics in throughout water column including sea surface and also seafloor, but the fate of microplastics in the ocean remain largely unknown. Sediment trap can be a useful tool for understanding sinking process of microplastics and estimate their vertical flux. Present study was conducted to identify the deposition characteristics and rate of microplastics, and estimate their deposition flux to bottom sediment in a semi-enclosed bay, Masan Bay, South Korea. A sediment trap was deployed 3 m above the sea floor in the middle of the bay from March 2019 to April 2020, and settled particles in collector tube of sediment trap was collected at monthly intervals for microplastic analysis. After organic matter removal and density separation processes, every plastic-like particles were analyzed using micro-Fourier transform infrared. The mean concentration and sedimentation rate of microplastics were 3.19 ± 1.52 n/g dry weight (1.35 – 6.20) and 67.09 ± 32.90 n/m^2/day (30.30 – 120.47), respectively. Correlation was not found between sediment accumulation rate and microplastic concentration. Microplastics accumulation rate was low in summer. Thermocline formed in the summer can limit the water circulation, preventing sinking of microplastics. High levels of microplastic accumulation rate was identified in October and November. Two big typhoons passing through the study area during this period may cause the redistribution of microplastics. Contrary to our expectation that microplastic level would increase after rainfall, there was no correlation between precipitation and microplastic accumulation rate. Based on the microplastic sedimentation rate, the sedimentation flux of microplastics in Masan Bay (70.9 km^2) was estimated to be 140 billion per month and 1.7 trillion per year. Low density polymers comprised a large proportion (> 80%) of total microplastics. This result could be due to vertical mixing of water, biofouling and aggregation. Fragments smaller than 300 µm were dominant. This study provides information about monthly variation in microplastic sedimentation and its contamination characteristics, which would be useful for understanding the processes related to microplastic burial.

01.08.12 Nanoplastics Reduce the Mitochondrial Toxicity of Rotenone in Developing Zebrafish

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Plastic toxicity has received global attention, but the toxicity pertaining to nanoplastics (NPs) remain under studied. Nanoplastics are continuously released into the environment causing threats to the wildlife and humans. In the presence of other contaminants these NPs may act as chemical carriers or modify the toxicities but has received less attention in the literature. Therefore, we investigated toxicity of polystyrene nanoparticles (Nano-PS) in the presence of pesticide rotenone, a potent mitochondrial toxicant. Zebrafish embryos at 6 hpf were exposed to Nano-PS (0.1 – 10 ppm) or rotenone (5ppb) or co exposed to a combination of both. Larvae exposed to only Nano-PS did not show significant changes in survival rates, but developmental deficits were detected. Larvae only exposed to rotenone showed developmental delays, reduced survival, and altered behavior. When combined, developmental deficits were lower than rotenone alone, exhibiting signs of toxicity rescue by NPs. This was correlated with the improved behavior observed in the co-exposed animals compared to Nano-PS and rotenone only exposures. In addition, lower Nano-PS bioaccumulation was detected in the co-exposed larvae. Interestingly, individual exposures to Nano-PS and rotenone decreased the mitochondrial coupling efficiency, while at co-exposures mitochondrial coupling efficiencies were improved. These data suggested that Nano-PS exposures would likely to have negative effects, but at combined exposures, rotenone may sorb to the surface of the Nano-PS and modify the toxic effects on an organism. The likelihood of an organism to expose to multiple contaminants are high in natural habitats and determining impacts on fundamental cellular physiological processes are critical.

01.08.13 Evaluating Microplastic Ingestion Across Trophic Levels in the Northeast Pacific Ocean

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The presence of microplastics in marine ecosystems has been documented in a wide range of locations and matrices around the world. Determining microplastic exposure across trophic levels is a crucial first step to understanding the potential toxicity of this contaminant and provides data that can be used in microplastic risk assessment efforts. However, there remain many places for which data does not exist regarding microplastic ingestion by local marine organisms, including large areas off the North American west coast. To address this evidence gap, we are investigating microplastic presence among organisms in remote locations in the Northeast Pacific Ocean for three separate projects. This includes an assessment of microplastics internalized by zooplankton caught within the Northern California Current (NCC) off the coasts of Oregon and northern California in 2019, as well as zooplankton retrieved from light traps in gray whale (*Eschrichtius robustus*) feeding grounds off the coast of Oregon. The occurrence of microplastics in sea otter (*Enhydra lutris*) feces is also being determined using samples collected in 2019 and 2020 from the Gulf of Alaska. Sample processing includes digesting organic material with potassium hydroxide, filtering down to 5-20 µm, and confirming a subset of potential plastics with FTIR spectroscopy. Preliminary data show that 30.1% of zooplankton captured within the NCC contained potential microplastics, with the majority of these being fibers (64%). Sea otter fecal samples contained similar microplastics to those found in zooplankton, with blue fibers accounting for the largest category of particles (27%), though further analyses are underway. Microplastic characteristics (size, shape, composition, weathering, etc.) are a key factor in determining their potential harmful effects on different types of organisms that ingest them. The results from these projects will provide up-to-date real-world data useful in risk assessment and lab studies that will increase understanding of the fate and impacts of microplastics in coastal ranges.

01.08.14 Transfer of Microplastics From Aquatic to Terrestrial Ecosystems

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Microplastics are important emerging contaminants in marine, aquatic, and terrestrial environments. As microplastic pollution increases, it is important to understand their occurrence, fate, and transport in these environments. Although the trophic transfer of microplastics within ecosystems has been studied, we lack empirical evidence of the trophic transfer of microplastics across ecosystems. This study aims to characterize a pathway of microplastic movement from aquatic to terrestrial ecosystems, using *Tachycineta bicolor* (tree swallows) as a model organism. Analysis of microplastics in water, sediment, insects, and lower digestive tracts of *T. bicolor* nestlings from three locations along the St. Louis River in Duluth, MN and Milwaukee River in Milwaukee, WI was conducted to determine microplastic movement from aquatic to terrestrial ecosystems. Modified versions of the *NOAA Marine Debris Program's Laboratory Methods for the Analysis of Microplastics in the Marine Environment* were used to remove organic material from samples for microplastic analysis. Plastic composition was identified with Fourier-Transform Infrared Spectroscopy (FTIR). Preliminary analysis found microplastics in sediment, water, and the lower GI tracts of *T. bicolor* from nine sites in Cedarburg, Milwaukee, and Sayner, Wisconsin. The presence of microplastics in the lower GI tract of tree swallow nestlings suggests that adult swallow feeding on aerial insects with aquatic immature stages was the source of microplastics. More detailed results will be presented at the SETAC conference.

01.08.15 Assessing the Toxicological Effects of Daphnia magna From Chronic Exposure to Polyethylene Nanoplastics and Natural Organic Matter

J. Brown, Oregon State University / Department of Engineering; B.J. Harper, S.L. Harper, Oregon State University / Department of Environmental and Molecular Toxicology

Plastic pollution is a major global concern that threatens the stability of aquatic ecosystems. The effects of micro and nanoscale plastics, with their high surface area to volume ratio, on freshwater species are still not understood. As one of the most commonly utilized plastic types, polyethylene (PE) nanoplastics chronic exposures present a model for assessing the long-term environmental effects of the presence of plastics in freshwater ecosystems. As an environmental indicator species and a species at the bottom of the food chain, *Daphnia magna* act as a model for environmental health when exposed to these plastics. In this study juvenile *Daphnia magna* were exposed to 25 ppm 200-999 nm PE nanospheres with and without the presence of 25 ppm *natural organic matter (NOM)*. To assess the effects of mixing, a set of exposures containing Daphnia media of the same size range and concentration of PE nanospheres were placed on a shaker table at low speed for the duration of the experiment. All exposures and controls (with or without NOM) were performed in triplicate and contained 5 individuals that were reared for a total of 28-days. Mortality, immobility, and reproductive output were assessed daily prior to feeding. All offspring were removed and placed into clean media during daily assessments and the number of offspring was recorded. After 28-days, the *D.magna* were removed from solution and imaged for growth assessment. Data collected showed a 40% survival for the *D.magna* exposed to both PE nanospheres and NOM after 28-days, compared to a 67% survival rate for exposures to PE where NOM was not present. There was no significant difference in survival rates between the NOM and *D.magna* media control groups. Shaking did not significantly impact survival. Daphnids exposed to media containing NOM, both with and without the presence of nanoplastics showed a significant increase in total neonate production. There was no significant effect on growth among any of the exposures. Additional investigation is necessary to further understand the chronic effects and modes of toxicity for a variety of different nanoplastics types on *D.magna* and other indicator organisms.

01.08.16 Influence of Particle Size on the Chronic Toxicity of Polymethyl Methacrylate (PMMA) Nanoparticles to Daphnia magna

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Understanding the impacts of nano-scale plastics on aquatic organisms is important in determining both the current and future risks to organisms. Polymethyl methacrylate (PMMA) is a transparent thermoplastic that is typically used as a lightweight and crack-resistant alternative to glass. We investigated the chronic toxicity of two sizes (25 and 50 nm) of PMMA nanospheres to *Daphnia magna* over a 28-day chronic exposure. Commercially available PMMA spheres were dialyzed for 1 week prior to experimentation to remove any manufacturing preservatives. Juvenile daphnids (1 week old) were statically exposed to either 5 or 50 mg/L of PMMA nanoparticles and their survival, growth and reproduction were monitored for 28 days with twice weekly solution changes. In addition, we investigated the behavior of these particles in simulated hard water used for the study through dynamic light scattering. The size distributions of each size particle in the simulated hardwater showed significant agglomeration of 25 nm, but not 50 nm particles (hydrodynamic diameters of 1975, 81 nm respectively). Following the 28-day exposure to each concentration and size of particle, only the highest concentration (50 mg/L) of PMMA particles with 25 nm diameters caused significant mortality in the adult daphnids over the 28-day exposure, while no other concentrations or particle sizes caused any significant mortality. Although the decreased survival in the high concentration of the 25 nm PMMA resulted in a significant decrease in total neonate production, no significant differences were found in the number of neonates produced per daphnid per day in any of the exposures. No changes in body length were identified at the end of the exposure. These results suggest that the primary particle size of PMMA nanoplastic spheres and their propensity to agglomerate may be important factors in determining their toxicity, especially when diameters are < 50 nm.

01.08.17 Spatial and Temporal Occurrence of Microplastics and Anthropogenically Impacted Materials in Black Rockfish (Sebastes melanops) Off the Coast of Oregon

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Microplastics (< 5mm diameter) are present in hundreds of marine and freshwater species. Understanding which species, the global spatial distribution, and what quantities microplastics are present across taxa is critical to understanding the potential impacts they could have on organisms, as well as to make possible the assessment of risk. We analyzed the gastrointestinal tract (GI) of wild Black Rockfish (*Sebastes melanops*) sampled off the Oregon coast for the presence of microplastics, with a specific focus on four different marine protected areas. Monitoring started at two of the Reserve sites in coastal Oregon in 2010 and restrictions started in 2012 and continued to be implemented until 2016. Suspected plastics or anthropogenically impacted materials were found in 93.1% of the Black Rockfish caught, present in fish from every site, in some cases at higher amounts than a positive control site closer to a coastal urban area. Fibers were the most prevalent morphology at 56.4% of debris items identified, with clear being the most abundant color. Plastic types such as polyethylene, styrene-ethylene butylene, and polypropylene were identified using Fourier Transform Infrared Spectroscopy. Additionally, ensuring the recruitment of viable larval and juveniles is pivotal to the health of adult fishes. For this reason, microplastic ingestion rates in the juvenile Black Rockfish from two Oregon estuaries were examined. The estuaries consisted of one marine protected reserve and one control site not protected from human activity, to investigate if there is a difference in microplastic ingestion rates between the two locations. The gastrointestinal tracts of the juveniles were removed and digested with potential microplastics then being run through the Fourier Transform Infrared Spectroscopy. Currently, clear and blue fibers were the most predominant of colors found and fibers were the most prevalent morphology. Black rockfish are both recreationally and commercially important fish to the state of Oregon and understanding the impact anthropogenic factors such as microplastics may have on them, and the implications of having plastics in marine reserves will be valuable for risk assessment, as well as future policy plans and actions.

01.08.18 Seasonal Stressors Impact the Combined Toxicity of Cadmium and Microplastics to the Benthic Invertebrate, Hyalella azteca

L. Zink, A. Hoveling, G.G. Pyle, University of Lethbridge / Biological Sciences

The inevitable introduction of microplastics to freshwater systems by domestic and commercial effluent results in increasing occurrence of microplastics in aquatic systems globally. While it remains unclear whether microplastics are toxic on their own, microplastics can interact with metals, which are ubiquitous in freshwater environments, allowing microplastics to serve as a vector for other known toxicants. Current research surrounding microplastics and metals are conducted in ambient conditions, not taking into account the ecologically relevant seasonal variability of real-world scenarios. In order to determine whether the seasonal variability of temperature and light cycle impacted the toxicity of microplastics and metals, *Hyalella azteca* were acclimated to seasonal conditions and subsequently exposed to microplastics, cadmium, or their mixture for either acute (96 hour) or chronic (42 day) timelines. Apical and behavioural endpoints were assessed throughout and following the experiments. Our work suggests that seasonal variability does impact the toxicity of these contaminants, highlighting the need to consider this natural variation when assessing ecologically relevant microplastic mixture toxicity scenarios.

01.08.19 The Type of Urban Stormwater Drainage Infrastructure Influences Microplastic Transport in Rivers

B. Dabney, D. Kashian, Wayne State University / Biological Sciences

Stormwater effluent may be a major pathway of microplastic contamination in rivers and streams. However, it is still unclear the role of drainage infrastructure (e.g. surface drains and pipes) on the transport of microplastics in freshwater systems. In this study, we measured microplastic concentrations at surface drains (pervious) and pipe (impervious) stormwater outfalls in the Clinton and Rouge River Watersheds in Michigan. The Clinton River watershed has more agricultural and forested areas and the Rouge River watershed has more residential and commercial developments. We also sampled upstream and downstream of the stormwater outfalls to determine the influence of outfalls on microplastic accumulation. At each sampling location (n = 60), three surface water and benthic sediment samples were collected, as well as water quality parameters.

Samples were digested and sediment underwent an additional 2-step density separation procedure. Microplastics were stained with Nile Red and identified under a microscope with fluorescence to obtain microplastic concentrations and morphology (sphere, fragment, fiber, or film). Results showed that fragments had the highest concentration in both water and sediment and were the better indicator of differences between sites. The highest microplastic concentrations were found mostly near pipe outfalls in both water and sediment. Surprisingly more microplastics accumulated at downstream locations in the more agricultural and forested watershed compared with the more developed watershed. This study suggests that land use characteristics from upstream sources and the type of drainage infrastructure are useful for understanding microplastics transport and may provide ecosystem managers the information required to design monitoring and remediation programs.

01.08.20 Quantifying Intertidal Microplastic Distributions: Implications for Monitoring and Invertebrate Exposure Assessment

C. Munoz, Radboud University / Environmental Science; P. Vermeiren, Radboud University / SIAM

Microplastic contamination in the intertidal of beaches and estuaries has been reported worldwide. Nonetheless, evidence of the fate of microplastics along and across the intertidal is often contradictory. When such conflicting evidence is used to inform monitoring campaigns, it increases uncertainty in resulting data. Moreover, the conflicting patterns hamper efforts in spatially explicit risk characterization of microplastic pollution to intertidal organisms. This project therefore aimed to guide sampling designs for monitoring of microplastics in the intertidal, and to quantify the exposure of invertebrates to microplastic pollution. In a first phase, a series of experiments was conducted that integrated recent methodological advances in microplastic extraction, quantification, and identification into a validated protocol that was applicable to organic-rich sediments with fine grain size. Pre-treatment with Fenton's reagent was efficient in reducing organic matter, and compatible with micro-Fourier Transform Infrared Spectroscopy (µFT-IR) for polymer identification. Density separation with $ZnCL_2$ in a Top Overflow Column (OC-T) obtained recovery rates > 90 % across polymers. Automated microscopic image analysis of Nile Red stained samples allowed quantification down to 125 – 66 µm, and > 90 % of validated particles to be determined as plastic polymers in both estuarine and beach samples. In a second phase, microplastic fate in the intertidal of sandy beaches and estuaries was examined, where the distribution across and along the intertidal related to dominant physical gradients including sediment grain size and structural complexity. Differences among sites were additionally influenced by proximity to anthropogenic sources. These results stress the importance of sampling with sufficient replication across the intertidal, and highlight the importance of local environmental covariates when comparing among sites. In a final phase, microplastic body burdens within dominant invertebrates were compared to sediment loads. Exposure landscapes to intertidal invertebrates were found to vary at local, within home ranges, and larger, among population, scales. Nevertheless, risk assessment needs to account for species-specific bioaccumulation rates to relate body burdens to sediment levels.

01.08.21 A Study on the Differences of Microplastics Distributions in the Surface Freshwater Collected by 100 and 355 Micrometer Nets

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Microplastics are recently considered anthropogenic pollutants, and researchers have dealt with the issues in various study realms. One of the arguments to describe the microplastic pollution was which net pore size should be employed. Generated microplastic quantity differences, which are naturally caused by different net sizes, have been stated by many researchers, but how much specific plastic distribution is overlooked in large net sizes is still insufficient. Therefore, the present study demonstrated the differences of microplastic distributions qualitatively and quantitatively between 100 and 355 µm nets. A gradual increase in microplastic abundance toward small size was observed in the 100 µm net samples, whereas the 355 µm net had no specific size distribution. The cumulative probabilities relating to the minimum Feret diameter of film and fragment were divided into three parts. It implied 96.7% potential underestimation in the 355 µm net and 67.3% of potentially overlooked particle numbers in the 100 µm net. The film, fiber, and fragment with seven polymers were discovered by the 100 µm net, but the few shape and polymer types were revealed in the 355 µm net. In this respect, the 355 µm net could not represent tracing microplastic origins and presuming bioaccumulation potentialities. The median values of numerical and mass abundances were 12.5 particles/m^3 and 35.5 µg/m^3 respectively, in the 100 µm net, and 0.4 particles/m^3 and 0.5 µg/m^3 respectively, in the 355 µm net. Whereas the distribution tendency along the flow direction by the 355 µm net was affected by irregulars discovered in the sampling analysis step, the 100 µm net showed the highest abundances in the lower sampling station of the river adjacent to the urban. Additionally, the ranking of mass abundances in this river comparing other Japanese rivers highlighted a remarkable difference at 27th in the 100 µm net and 9th in the 355 µm net. In conclusion, the 100 µm net revealed completely different distributions by the 355 µm net and was recommended, but the proper sampling method should be employed through establishing strategic research plans.

01.08.22 Microplastics and Plastic Stabilizers in the Surface Water of the St. Lawrence River, Estuary and Gulf

K. Crampond, University of Quebec, Rimouski / ISMER; Y.D. Soubaneh, University of Quebec, Rimouski / Biologie, Chimie et Géographie; Z. Lu, University of Quebec, Rimouski / Institut des Sciences de la Mer de Rimouski; N. Toupoint, Merinov

Microplastics (MPs) and plastic-associated chemicals are of great environmental concern due to their persistent, bioaccumulative, and toxic properties. Although "plastic manufactured items" have recently been listed as toxic substances in the Canadian Environmental Protection Act, the status of MPs and plastic-associated chemicals in The St. Lawrence ecosystem remains largely unknown. The St. Lawrence River, Estuary and Gulf (SLREG) host a suite of unique ecosystems but also constitute a vector of contaminants to the Atlantic Ocean. The current lack of data on microplastics pollution in this region impedes understanding its effects on this ecosystem. The objectives of this study were to (1) quantify and characterize the MPs in the surface water (n=32) of SLREG; (2) measure the concentrations of plastic stabilizers including UV absorbents and synthetic antioxidants in the surface water samples (n=77), and (3) investigate the correlations between the levels of MPs and target plastic stabilizers in the surface water of SLREG. The preliminary results showed a decreasing trend of the abundance of MPs from the river to the estuary and the gulf. The highest abundance of MPs was found at the site downstream of Montreal and Quebec city with 43-49 particles/m^3 and the MPs decreased to 8-14 particles/m^3 at the gulf. The characterization of MPs using micro-infrared (µFTIR) and Raman spectroscopies and the plastic stabilizers analysis of in water are currently underway. This study will fill critical knowledge gaps pertaining to levels and profiles of MPs and plastic stabilizers in the SLREG. Knowledge gained through this project will provide new insights into exposure risks and the distribution/transport of these contaminants in the SLREG.

01.08.23 Microplastics in the Terrestrial Environment: Is There a Real Threat and Risk?

I. Bamgbose, Gradient

In recent years, there has been an increase in scientific and societal concerns about microplastics (MPs) in the terrestrial environment, in part due to ongoing increases in the production of plastics, application of plastic products, and general disposal by the general population. Based on a review of current literature of MPs in the terrestrial environment, there is

(1) a lack of critical evaluation of the current research trends related to the types and concentrations of MPs detected in terrestrial biota versus the types and concentrations of MPs and study organisms used in laboratory studies of ecotoxicological effects; and (2) inconsistencies between the particle types, size ranges, and concentrations of MPs used in laboratory tests and those measured in the environment. The objective of the poster is to assess the weight of evidence for MP biological effects in soil, biota, and other terrestrial matrices.

01.08.25 Microplastic Concentrations in Soil and Earthworm Samples From Central and Northern Virginia

T. Bustamante, University of Mary Washington; T.E. Frankel, University of Mary Washington / Earth and Environmental Sciences

Microplastics, defined as plastic particles under 5mm in size, have become a contaminant of concern in recent years. These plastics have been shown to leach and transport many chemical pollutants as well as be consumed by a variety of organisms. While most of the research on microplastics has been done in aquatic settings, the study of terrestrial microplastics is novel. In order to better understand terrestrial microplastics, this study quantified concentrations in soil and earthworm samples collected in central and northern Virginia. Soil and worms were collected from sixteen sites between Chesterfield county and Fredericksburg. Microplastics were extracted from these samples via a combination of organic matter digestion and density separation. Samples were then vacuum filtered, and the resulting filter paper was visually inspected for plastics under a dissecting microscope. Preliminary results from four of our sites show that microplastics are indeed present in soils and range in concentration from 0.03-0.64 particles/gram of soil. The highest concentration comes from a more urbanized area, providing some evidence that more microplastics are found in areas of higher human activity. These results show that while microplastics are present in soil from Virginia, they are nowhere near the concentrations that have been found in other areas of the world such as China. Future research aims to finish processing all samples and create a heatmap using the kriging model.

01.08.26 Microplastic Particles in Waste Water From Washing Clothes With Two Types of Detergents

P. Zaragoza-Pérez, P. Ramirez Romero, W. Alvarado-Aguilar, L.J. Sánchez Hernández, J. Gutiérrez-Pacheco, Universidad Autonoma Metropolitana Iztapalapa / Hidrobiologia

Today, the so-called "fast fashion" industry has substituted cotton and wool fibers with synthetic (polyester, nylon, polypropylene, etc.) ones. These materials break down, during washing, into microplastics which are transported in the wastewaters. A portion will settle and separate in the wastewater treatment plants but others will reach the environment. Previous works have studied the fibers produced when washing clothing items, therefore the objective of the present work was to evaluate the presence and characteristics of microplastic particles (MPP) produced by the garment washing using different types of detergents. A survey was done to identify the most popular liquid and powder detergents as well as the fabric softener. Also, to find out if people follow the manufacturer's instructions. Twelve samples were obtained from washing clothes with the most popular liquid detergent and, twelve more with powder detergent. In both cases, a fabric softener was used. 10L were filtered through a couple of Standard Testing Sieves (N 150 and N 35). Fibers were separated and the remaining particles were counted and characterized with the help of a digital microscope. Up to 61 MPP/L were found and there was no difference if clothing was washed with powder or liquid detergents ($p>0.05$). 50% of the particles were made out of natural cotton and the rest of synthetic materials which still need to be confirmed through chemical analysis. Particle sizes varied from 4 um to 1380 um. Like in other types of environmental samples, black was the most abundant color (60%).

01.08.27 Age-Dependent Transgenerational Responses in Daphnia magna Exposed to Polyethylene Microplastic Fragments Containing Benzophenone-3 Additive

C. Kim, J. Song, Korea University; J. Jung, Korea University / Environmental Science and Ecological Engineering

Microplastic (MP) pollution is a rising concern in the aquatic environment. Due to its small size, MPs have the potential to be ingested by organisms and induce toxic effects such as physical damage and oxidative stress. Furthermore, MPs may exert additional toxicity by leaching chemical additives. Since plastics are persistent in the environment, organisms are exposed to MPs over multiple generations. Life-long intergenerational exposure to MPs can exacerbate toxicity or trigger transgenerational plasticity in organisms and consequently alter species population. Therefore, multigenerational scale experiments are required to better predict the ecological risk of MPs in the aquatic environment. The aim of this study is to evaluate age-dependent transgenerational effects of polyethylene MP fragments (16.68 ± 7.04 m) containing benzophenone-3 (2.89 ± 0.20% w/w) on Daphnia magna over four generations (F0–F3). Neonate (< 24 h old) and adult (5 d old) daphnids were exposed for 21 days to identify the effect of age on transgenerational plasticity. Growth (body length of mother and offspring) and reproduction (days to the first brood, total number of offspring) parameters were measured for each generation to evaluate transgenerational effects.

01.08.28 Assessing the Presence and Concentration of Microplastics in the Gizzards of Virginia Waterfowl

T. Bustamante, University of Mary Washington; T. Duong, University of Mary Washington / Earth and Environmental Sciences; R. Gunraj, A. Dolby, University of Mary Washington; T.E. Frankel, B. Odhiambo, University of Mary Washington / Earth and Environmental Sciences; J. Asper, University of Mary Washington

Microplastics are defined as plastic fragments smaller than 5mm which originate from sources such as manufactured pellets, personal care products, and the breakdown of larger plastic items. They have become a ubiquitous water pollutant in recent years, and while a substantial amount of research on their impacts on marine ecosystems has been conducted, the presence of microplastics in freshwater systems and organisms remains less understood. In this study, we assessed the presence and concentrations of microplastic particles in the gizzards of the Canada Goose (*Branta canadensis*), Longtailed Duck (*Clangula hyemalis*), Ringneck Duck (*Aythya collaris*), Mallard (*Anas platyrhynchos*), and Goldeneye Duck (*Bucephala clangula*) hunted in the Piedmont and Coastal Plain of Virginia. Gizzards were bisected, then their contents were removed for analysis. Internal gizzard contents were digested in 30% hydrogen peroxide with an iron catalyst, then were density separated in a NaCl saline solution to isolate microplastics. Samples were then visually inspected under a dissecting microscope. After laboratory contamination was taken into account, 53.6% of gizzards contained microplastics. Samples ranged in concentration from 0 to 1.75 plastics/gram of gizzard material. While concentrations did not differ between sex and location, diving ducks had significantly higher microplastic concentrations than Canada Geese. The raw number of microplastics between the two groups was the same. This result may be due to differences in the diet between diving ducks and Canada Geese. These results provide evidence that freshwater species of waterfowl not only consume microplastics, but also retain them in their digestive tracts. Results were fairly high compared to studies assessing birds in more remote areas, suggesting the level of urbanization in our sites led to these results. As microplastics continue to release into the environment, more organisms, such as these waterfowl, will consume these plastics and potentially suffer toxicological consequences.

01.08.30 Assessing the Presence and Concentration of Several Anthropogenic Pollutants Near the Crow's Nest Natural Area Preserve (Stafford, VA)

C. Willmore, A. Tomba, T.E. Frankel, University of Mary Washington / Earth and Environmental Sciences

Various anthropogenic pollutants have been identified as contaminants of concern for freshwater environments. Microplastics (fragments < 5mm) have been widely detected in aquatic and terrestrial environments; while their large-scale effects are not well defined, unintended consumption or enmeshment of particles in gill structures have been observed in organisms. Excess nutrients such as nitrogen and phosphorus have been shown to cause rapid algae blooms which, subsequently, generate hypoxic or anoxic conditions as they die and decay. The Crow's Nest Natural Area Preserve (Stafford, VA) accounts for over 60% of all the marshes in the county, including over twenty miles of stream, wetland, and riparian buffer that serve as a habitat for many migratory bird and freshwater teleost species. As little research has been done to assess the above pollutants near this ecologically important location, this study examined the presence and concentration of microplastics, nitrate, orthophosphate, and total phosphorus in both sediment and surface water samples collected at six sites surrounding the preserve. To assess the presence and concentrations of microplastics in sediments, samples were obtained weekly using 2" stainless steel coring tubes. Samples were dried at 90^{o}C for 24h, after which three 100g subsamples were digested using wet peroxide oxidation, density separated using NaCl, and the number and type of microplastic particles quantified under fluorescent microscopy with nile red stain. Surface water samples were collected weekly from the same locations and two WS750 samplers were used to obtain surface water 1 and 3hrs after rain events. Water samples were assessed for the nutrients nitrate, orthophosphate, and total phosphorus using an Evolution 260 Bio UV-Vis spectrophotometer. Sediment samples were obtained from each site weekly using a Van Veen grab sampler, extracted using the Mehlich 3 technique, and analyzed for nutrient concentrations. Analyses of surface water, stormwater, and sediments are expected to yield varying levels of microplastics varying with flow conditions. It is expected that N and P levels will be higher in sediments than in surface waters and will also vary depending on rainfall events. Assessing these pollutants will provide vital information regarding the health of this important ecological landmark and will serve as support for further examination of their spatiotemporal presence in Chesapeake Bay tributaries.

01.08.31 Transgenerational Effects of Polyethylene Microplastic Fragments Containing Benzophenone-3 Additive on Daphnia magna

J. Song, C. Kim, Korea University; J. Jung, Korea University / Environmental Science and Ecological Engineering

Microplastics (MPs; < 5 mm) are a growing concern due to their small size, wide distribution, and bioavailability. Furthermore, chemical additives (e.g. photostabilizer, antioxidant, and flame retardant) incorporated with MPs can cause additional harmful effect to aquatic organisms. In this study, transgenerational effects of polyethylene MP fragments (16.68 ± 7.04 μm) with and without benzophenone-3 additive (2.89 ± 0.20% w/w) on *Daphnia magna* were investigated across four generations (F0-F3). Histopathology, embryonic development, somatic growth (body length of adults and offspring), and reproduction (number of offspring per female) were measured to evaluate the transgenerational effects. In addition, DNA methylation was determined to identify epigenetic mechanism in transgenerational effects.

01.08.32 Analysis of Microplastics in the Gastrointestinal Tracts of Odontocetes in the Southeast Region of the United States

J. Courville, Jacksonville University / Marine Science Research Institute; L.B. Sonnenberg, Jacksonville University / Department of Chemistry; R. Borkowski, Jacksonville University / Department of Biology & Marine Science; G.K. Bielmyer-Fraser, Jacksonville University / Chemistry

Globally, microplastics (< 5 mm in diameter) comprise approximately 92% of all plastic marine debris. Despite this extensive proportion, the subject of microplastics in marine mammals is vastly understudied. An increasing body of research in recent years is contributing to the emerging documentation of the microplastic profile of odontocetes living in the coastal Southeastern region of the United States. This study includes the following specimens: 15 bottlenose dolphins (*Tursiops truncatus)* stranded in Texas, Alabama, and Florida with one pygmy sperm whale (*Kogia breviceps*) and one pantropical spotted dolphin (*Stenella attenuata*) obtained from Southern Florida. Contents found in the gastrointestinal tracts, namely, three stomach chambers and a portion of the intestinal tract, were subjected to a laboratory procedure to isolate existing plastics. This process involved washing and sieving the material followed by filtrating and separating the biological matter to achieve size fractions between 125 μm and 5 mm. The suspected microplastics were analyzed by physical characteristics under a stereomicroscope and polymer classifications verified via Fourier Transformed Infrared Spectroscopy. It is hypothesized that each specimen will contain an array of microplastics with physical characteristics distinguished by the following categories: polymer type, quantity, size, shape, and color. Documenting and understanding the microplastic content in both deep diving and shallow diving odontocetes will strengthen the current knowledge of microplastic loading. This research advances further exploration of associated risks of microplastics in odontocetes within the United States and worldwide.

01.08.33 The Fragmentation of Clothing Fibres Varies by Polymer and Environment

B. Charlesworth, University of New South Wales / Biological, Earth and Environmental Sciences

Plastics are a global contaminant found in all environments, where they are thought to cause significant social, economic and ecological impacts. The quantities of fibres found as litter has increased by 450% over the last 70 years. Consequently, fibres now constitute 65% of all sampled debris. These fibres are shed into the drainage pathway when clothes are worn and washed. To mitigate this problem, the public, industries and governments are trying to replace durable, virgin plastic fibres with less durable natural fibres, however, the rate at which these fibres fragment in the environment is currently unknown. Here, I developed a novel apparatus and method to deploy plant, animal and plastic fibres in the environment and investigate their fragmentation over time. Using this method, I demonstrated that the fragmentation of fibres varies by location, environment (marine vs terrestrial), exposure to sunlight and type of polymer. Experimentally determining the rate at which these fibres fragment is useful to understand their transport and fate in the environment, as well as the threat they pose to wildlife through ingestion and toxicity. The hope is that this information, combined with future work, can be used to alter the way we make and consume clothing to ultimately reduce pollution.

01.08.34 Microplastic Menace? Exploring the Potential Effects of Microplastics on Early Life Stages of Freshwater Mussels

Y. Kudla, University of Guelph / School of Environmental Sciences; P. Gillis, C.J. Bennett, J. Salerno, Environment and Climate Change Canada / Aquatic Contaminants Research Division; R.S. Prosser, University of Guelph / School of Environmental Sciences

Plastic debris polluting our waterways has been a concern for decades. Recently increased attention has been placed on microplastics (MPs) contamination of aquatic ecosystems. These small particles of plastics (< 5 mm) have been observed in marine and freshwater ecosystems all over the Earth. Although effects of MPs ingestion on important marine invertebrate species have been demonstrated, little is known on the effect of microplastics on freshwater species. To date, freshwater studies have focused on the presence and/or concentration of microplastics in surface waters, but in order to assess their risk, there is a need to compare the environmental concentrations of microplastics to concentrations that cause adverse effects. Freshwater mussels are a group of filter-feeding organisms that have experienced a global decline due to habitat destruction and declines in water quality and they are under-represented in microplastics research. In this study, standard methods were used to conduct acute (48 h) toxicity tests with glochidia (larvae) of *Lampsilis fasciola*, a species of special concern. Tests were performed with pristine microplastic spheres of varying polymer types and sizes. Polystyrene (6 and 90 µm), polyethylene (75-90 µm), and cellulose acetate (1000 µm) spheres were used in treatment concentrations spanning 50-300,000 MP/L. Glochidia viability (i.e., the ability to close valves) was used as a surrogate for survival. L. siliquoidea glochidia were insensitive to each type of microsphere tested with effect concentrations being >300,000 MP/L for polystyrene and polyethylene and >1000 MP/L for cellulose acetate. Tests were also conducted with juvenile *Lampsilis siliquoidea* with similar concentration, polymer types and sizes. This data will help inform the risk assessment of microplastics to freshwater biota. The recent focus has been on assessing the presence and/or concentration of microplastics in freshwater systems. In order to assess their risk, we must be able to compare the concentration in the environment to the concentration that will cause an adverse effect.

01.09 Fate and Effects of Metals: Biogeochemical Perspective

01.09.01 Assessment of Bioavailable Mercury in Soils and Sediments

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The fate and behavior of mercury (Hg) in sediment environments is dependent on the chemical and physical form of the Hg and the sediment geochemistry. Some forms of Hg are more available for chemical and biological transformation to methylmercury (MeHg), which can bioaccumulate and biomagnify in the food web. The goal of this research is to develop a simple laboratory assay to experimentally assess biologically available Hg using an assay that identifies the MeHg production potential of soils and sediments. Hg availability and methylation potential was evaluated in both stirred slurries and in stagnant jar microcosms for samples collected from freshwater, marine, and terrestrial floodplain sediments. Microcosms included both native sediments and sediments amended with lactate to aid microbial degradation, ferric iron to encourage iron reducing conditions and sulfate to encourage sulfate reducing conditions. Additionally, a reference sediment was amended with specific Hg forms of HgS and $Hg(NO_3)_2$. THg and MeHg were measured in both sediments and porewater at two time periods designed to maximize MeHg formation. Porewater concentrations were measured via DGT devices, and in filtered water. Sulfide, oxygen, redox conditions, and other biogeochemical conditions measured in the microcosms were used to identify the best indicators of methylation potential and develop conceptual models to predict methylation. Results show that sediment/porewater partition coefficient (K_d) which indicates the amount of dissolved $Hg^{2+}$was a good indicator of methylation potential, whereas sulfide and redox measurements and the sulfate/iron/lactate amendments were less strongly correlated with methylation. Another set of experiments were designed to investigate the effect of sediment size fractions and Hg speciation (assessed separately by XAS) on methylation potential. One freshwater river sediment and one marine sediment were selected, and they were separated into 3 size fractions (bulk, < 0.5 µm, and 2-45 µm fractions). Pure cultures of the model MeHg methylator ND132 Desulfovibrio Desulfuricans were exposed to different Hg sediment size fractions. MeHg production was measured in the different size fractions after t = 0, 12 and 24 h and related to the bulk assay methylation potential.

01.09.02 Methylmercury Production and Degradation in the Water Column of the Hells Canyon Reservoirs, USA

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Methylmercury (MeHg) is a highly toxic form of mercury (Hg) that can bioaccumulate in fish tissue. Methylmercury is produced by anaerobic bacteria, many of which are also capable of MeHg degradation. In addition, demethylation in surface waters can occurs via abiotic sunlight-mediated processes. The goal of this study was to understand the relative importance of microbial Hg methylation/demethylation and abiotic photodemethylation that govern the mass of MeHg within an aquatic system. The study location was the Hells Canyon Complex (HCC) of three reservoirs on the Idaho/Oregon border, USA that has fish consumption advisories due to elevated MeHg concentrations. Our study utilized stable isotope addition incubation experiments to trace MeHg formation and degradation within the water-column of the reservoirs using multiple techniques. Monitoring data of MeHg concentrations measured in the water column of the reservoirs were used to compare the MeHg production rated identified in the incubations experiments with the actual mass of MeHg formed in the reservoirs over time. The results showed relatively good agreement between the different methods used to identify MeHg production. Overall, the rates of MeHg production and degradation within the water column were relatively low ($< 0.07\ d^{-1}$) but were sufficient to account for most of the MeHg observed with the system. Most MeHg production within the water column appeared to occur in the spring when much of the water-column was in the processes of becoming anoxic. In the surface waters, rates of photodemethylation were relatively large (up to $-0.25\ d^{-1}$), but quickly decreased at depths deeper than 0.5m below the surface.

01.09.03 The Effects of Sediment Geochemistry on Methylmercury Bioaccumulation in Invertebrates in the Minas Basin, Bay of Fundy

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Methylmercury (MeHg) is a toxic contaminant that readily bioaccumulates and biomagnifies in food webs, and negatively affects organism and ecosystem health. Impacts on ecosystem health from MeHg have been extensively studied in freshwater ecosystems however much less is known about MeHg retention and biomagnification in coastal ecosystems. Intertidal invertebrates are abundant in estuaries and are critical prey sources for migratory birds and marine fish, and thus determining the uptake of MeHg by intertidal invertebrates is essential for determining MeHg exposure in higher trophic level organisms in the food web. This research quantifies MeHg levels in invertebrates and relates bioaccumulation of MeHg to changes in sediment porewater concentrations of sulfate, dissolved organic matter, and mercury in five coastal estuarine locations in the Minas Basin, Bay of Fundy. The formation of MeHg by sulfate-reducing bacteria during the reduction of sulfate to sulfide suggests that systems with sulfate loading may have increased MeHg concentrations in sediments. However, binding of Hg with sulfide may reduce bioavailability of these complexes to methylating bacteria. Dissolved organic matter (DOM) may reduce the uptake of MeHg by invertebrates due to its size, however DOM may also increase MeHg production by acting as an energy source for methylating bacteria. We hypothesize that bioaccumulation of MeHg in invertebrates (with a focus on Corophium volutator, Ilyanassa obsoleta, and Polychaeta) will be greater in sediments with increased sulfate relative to DOM. To assess the effects of sediment geochemistry on MeHg bioavailability to invertebrates, organism MeHg concentrations were compared to DOM concentration, sulfur speciation, and mercury speciation in porewater and sediment. This research provides quantitative data on MeHg bioavailability in the Minas Basin which can be used to protect both ecosystem and human health. By identifying areas that are at greater risk for increased MeHg production this research sheds new insight on the health of ecosystems critical to migratory birds, coastal fisheries and many industries in Atlantic Canada.

01.09.04 Temporal and Spatial Trends of Mercury (Hg) Isotopes in Northern Pike From Lakes Under the Influence of the Flin Flon, Manitoba, Canada, Metal Smelter

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The Flin Flon smelter in northern Manitoba (Canada) began operating in 1931 for the refinement of zinc and copper ores. Until its closure in 2010, the smelter emitted between 1.4-19.9 t/yr of mercury into the atmosphere, potentially contaminating surrounding lakes. Monitoring of mercury (Hg) in water bodies of Northern regions is particularly important because their fisheries are a substantial food source to local communities. Regular assessment of food web and sediment samples from the region by Environment Canada has shown that total mercury (THg) and methylmercury (MMHg) are elevated, however have unexpected spatial and temporal patterns when compared directly with smelter activity. Our investigations of Hg isotopes is providing new insights into Hg pathways that could assist with explaining anomalies in spatial and temporal trends and improve mercury monitoring. Our study investigated five lakes in the Flin Flon area from 2008-2013 (range 4.5-110 km from smelter) and focused on top predator species, northern pike. Multi-collector Inductively Coupled Plasma Mass Spectrometry (MC-ICP-MS) was used to measure Hg isotope ratios. Both Δ^{199}Hg and Δ^{201}Hg from northern pike in these lakes indicate high levels of Mass Independent Fractionation (MIF). Analysis of Variance (ANOVA) of Δ^{199}Hg data from pike show a significant difference over three years ($p< 0.001$) and a significant difference in the signature between the lakes ($p< 0.001$). There was no obvious directional or distance to source trend for MIF signatures in the lakes, but McLurg Lake and Meridian Lake showed more variation both from the other 3 lakes and between years sampled. Mass Dependent Fractionation (MDF) characterized by ∂^{202}Hg values in all the lakes vary from -0.93 to -0.18 with a noticeable increase in ∂^{202}Hg over 3 years. ANOVA of ∂^{202}Hg data from pike show a significant difference over the years ($p< 0.001$) and a significant difference between the lakes ($p< 0.001$). Our findings suggest that each lake has a characteristic Hg isotope signature, which changes in the same fashion over the observation period. We can use this Hg isotope data to further understand photochemical degradation of Hg in these lakes over time or to assesses if these lakes are being exposed to newer deposits of atmospheric Hg, such as another global source or re-emission from the watershed. By studying changes in Hg isotope ratios we hope to explain differences in mercury concentrations of organisms within these lakes.

01.09.05 Sources and Sinks of Total Mercury and Methylmercury in the Kongsfjorden, Svalbard

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The arctic fjords have undergone rapid environmental changes over the past decades, such as increases in glacial discharge, sea ice loss, overland runoff, and river discharge, which may modify transport and transformation rates of mercury (Hg) in seawater. Despite this, distribution and mass flux of Hg in the arctic fjords have been understudied. We investigated total Hg (THg) and methylmercury (MeHg) concentrations in seawater of the Kongsfjorden, Spitsbergen, in the Svalbard archipelago in the summers of 2016 and 2017. THg and MeHg were also measured in the exterior sources, such as the Bayelva River, Lake Solvatnet, drift ices, multiple streams, and rainwater. The mass budgets of THg and MeHg were estimated using the steady-state Hg transport model (SERAFM) calibrated with field-monitored Hg concentrations. The model predicted that concentration was 1.1 pM for THg and 86 fM for MeHg in seawater, corresponding with calibration errors of 1.2% for THg and 21% for MeHg. The mass budget showed that major sources of THg are Atlantic water and glacier discharge, and those of MeHg are Atlantic water and in situ methylation in the shallow halocline water. These results agreed to the distribution patterns of Hg, as the highest THg was found in front of the tidewater glacier (Kronebreen) and that of MeHg was found in the stratified surface water of the river mouth. Although a steady-state model was not completely modified to represent future warming conditions, a preliminary simulation with a 900% increase in glacier discharge rate resulted in a 25% increase of the MeHg concentration in the trophic level 4 fish, and a 30% increase in dissolved organic carbon concentration resulted in 35% decrease of MeHg concentration in the same fish. The associated parameters with climate warming should be carefully monitored to understand the fate of Hg in the current and future conditions of the arctic fjords.

01.09.06 Complex Copper Transformations in Sediments Affect Toxicity

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The chemistry of copper (Cu) in sediments is complex and not well described at low concentrations. We evaluated the transformation processes of Cu added to freshwater sediments under suboxic and anoxic conditions. Freshwater sediments with varying characteristics were spiked with 30 or 60 mg Cu kg–1 and incubated under a nitrogen atmosphere. After 28 days, a subset of each treatment was amended with organic matter (OM) to promote anoxic conditions and evaluate its effects on Cu speciation. The addition of OM triggered a shift from suboxic to anoxic conditions, and sequential extractions showed that the Cu speciation accordingly shifted from acid soluble/reducible to oxidizable fractions. EXAFS spectroscopy revealed that CuS, or a similar phase, to be predominant in all anoxic samples except for Spring Creek 30 mg kg–1, where Cu(I)-thiols was suggested to be predominant. Copper was also reduced in part to CuS under suboxic conditions, although around 20% persisted as Cu(II) complexed to humic acid. In coarse sediments (i.e., Spring Creek), however, most of the Cu remained as Cu(I)-thiol and Cu(II)-humic acid. XANES, Scanning Electron Microscopy, and Raman spectroscopy confirmed covellite and chalcopyrite to be predominant in nearly all anoxic samples. Toxicity tests showed that survival of H. azteca in anoxic surficial sediments and D. magna in the overlying water was similar to controls, while it was significantly lower in suboxic treatments ($p = 0.001$ and < 0.001, respectively). In summary, anoxic conditions in freshwater sediments trigger a complete transformation of Cu speciation to sulphide minerals, reducing its toxicity.

01.09.07 Tools for Understanding Exposure to Bioavailable Metals in Sediment Bioassays: Collection and Use of Porewater Data

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The US Environmental Protection Agency *Procedures for the Derivation of Equilibrium Partitioning Sediment Benchmarks (ESBs) for the Protection of Benthic Organisms: Metal Mixtures (Cadmium, Copper, Lead, Nickel, Silver and Zinc)* equilibrium partitioning approach causally links metals concentrations and toxicological effects; it applies to sediment and porewater (i.e., interstitial water). The evaluation of bioavailable metals concentrations in porewater, using tools such as the biotic ligand model, provides an advancement that complements sediment-based evaluations. However, porewater characterization is less commonly performed in sediment bioassays than is sediment chemistry characterization, due to the difficulty and expense of porewater collection, as well as concerns about interpretation of porewater data. This evaluation discusses the advantages and disadvantages of different extraction methods for collection of porewater for analysis of metals and bioavailability parameters as part of sediment bioassays, with a focus on peepers and centrifugation. Comparisons of paired exposure data from bioassay studies indicate that metals porewater concentrations collected via centrifugation tend to be higher than those collected via peepers. However, centrifugation disrupts the redox status of the sediment and metals concentrations can vary greatly based on centrifugation conditions. Data to compare concentrations of peeper- and centrifugation-collected bioavailability parameters are much more limited but indicate smaller differences than those seen for metals. While porewater can be collected using peepers without altering the redox status, the small volume of porewater that can be collected by peepers is enough for metals analysis but insufficient for analysis of all metals bioavailability parameters. Given the benefits of collecting porewater for analysis of metals via peepers, this method may be very useful for understanding metals toxicity in bioassays, even when porewater for other bioavailability parameters must be collected by centrifugation.

01.09.08 Comparison of Multiple Linear Regression and Biotic Ligand Models for Predicting Zinc Toxicity to Freshwater Organisms

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As part of a Cooperative Research and Development Agreement (CRADA), the U.S. Environmental Protection Agency (USEPA) and several metals associations are collaborating to develop a bioavailability-based modeling approach that will be used to develop updated aquatic life ambient water quality criteria (AWQC) for metals. The bioavailability models being considered are the biotic ligand model (BLM; a quasi-mechanistic model) and multiple linear regression models (MLR; an empirical model). Biotic ligand models for zinc in freshwater have previously been published, while MLR models for zinc were developed in support of this initiative. Zinc MLR models were developed for three groups of aquatic life (algae, invertebrates, and fish), with both species-specific models and pooled-species models evaluated. Performance of the zinc BLM and MLR models were then compared based on several metrics developed at a 2017 CRADA workshop, which were subsequently published. In addition, we considered modifications to some metrics, as well as some additional metrics, for assessing both model performance and model validation. In general, the zinc BLM and MLR models were observed to perform comparably. Both model types were then used to develop aquatic life criteria following USEPA guidelines for AWQC development to demonstrate how criteria derived from each model type compare over varying bioavailability conditions.

01.09.11 Using Biofilms As Bioindicators of Metal Contamination in Stream Ecosystems: Towards the Development of a Predictive Accumulation Model

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Assessing metal contamination in aquatic ecosystems can be complex. Indeed, several environmental factors can lead to rapid fluctuations in water concentrations, especially in small streams. Concerning metals, these factors can modulate their concentration as well as their speciation and bioavailability. Because multiple measurements are required for an adequate representation of that variability, biomonitoring is an interesting approach that provides an integrated response to environmental contamination and metal bioavailability. Stream biofilms are very useful and complementary to water quality monitoring because of their accumulation capacities: they integrate the inherent variability of element concentrations in aquatic environments. Moreover, biofilms have valuable potential for biomonitoring: they are sessile, ubiquitous and at the base of the trophic chain. The objective of the present study was to evaluate the potential of biofilm metal content as a biomarker of metals in streams impacted by mining activities and to identify the key parameters (pH and cation competitor concentrations) that modulate metal accumulation in biofilm. Seven sampling campaigns in the Nunavik region (northern Quebec) as well as in the Sudbury area (southern Ontario) were conducted to study the relationships between various parameters such as metal speciation and metal accumulation by the biofilms. The results of metal accumulation were highly consistent from year to year, and region to region. Significant linear relationships were determined between calculated free metal ion concentrations of copper and nickel and biofilm metal contents. However, at low pH values and high hardness, biofilm showed lower metal accumulation than expected even for high free metal ion concentrations. This is not surprising as protons and hardness cations can lead to a protective competition effect on biological membranes resulting in lower internalised concentrations. If we consider that biofilm metal content was consistent over a large geographical scale despite biological/taxonomic differences and a variety physico-chemical characteristics, biofilm bioaccumulation seems to be a highly robust water quality indicator of metal contamination. The next step is to develop a Biotic Ligand Model to predict biofilm metal content in natural waters. This biofilm-based approach using bioaccumulation as a biomarker shows good potential, which would be greatly helpful as an environmental management tool.

01.09.12 The Presence, Distribution, and Concentration of Trace Metals in the James River Near a Coal-Burning Repository

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The Chesapeake Bay is one of the largest estuary systems on the east coast of the United States, containing several coal-burning power stations located along its waterways. One of the largest forms of industrial waste, coal ash, is primarily produced by coal-burning power stations and is known to be heavily enriched with trace metals that are at high risk for leaching into surrounding aquatic environments. Few studies have examined the concentrations of trace metals within a tributary of the Chesapeake Bay, the James River watershed, and the implication of a coal-burning repository located in its upper reaches. Thus, the goal of this study was to assess the spatial and temporal distribution of trace metals in water and sediments in the vicinity of the Chesterfield power station (Richmond, VA) within the James River. Water and sediment samples (grab and core) were collected upstream (6 sites), midstream (9 sites), and downstream (13 sites) from the Chesterfield power station. Sediments samples were digested in aqua regia prior to trace metal analysis. Both water and sediment samples were analyzed using ICP-OES (inductively coupled plasma optical emission spectroscopy) for the concentration of twelve trace metals: Al, As, Cd, Ca, Cr, Cu, Fe, Pb, Mg, Mn, Se, and Zn. Preliminary results of water and grab samples show elevated concentrations of trace metals downstream and behind the power station near the Dutch Gap Conservation Area. Concentrations of cadmium in the water (0.005-0.017 ppm) exceeded the EPA's Maximum Contaminant Level's for drinking water. Concentrations of trace metals (As, Cd, Pb, Se) were found higher in the sediment core collected midstream to the power station compared to cores collected upstream and downstream. Complete water and sediment cores sample analyses will provide a clearer picture of loading at the study site as well as the spatial and temporal variability of trace metal contamination. This study will provide important information regarding the potential impacts of coal-burning repositories on the presence and mobilization of trace contaminants within aquatic ecosystems.

01.09.13 Sediment Toxicity Data and Excess Simultaneously Extracted Metals From Field-Collected Samples: Comparison to United States Environmental Protection Agency Benchmarks

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US Environmental Protection Agency (USEPA) *Procedures for the Derivation of Equilibrium Partitioning Sediment Benchmarks (ESBs) for the Protection of Benthic Organisms: Metal Mixtures* are based on the principle that metals toxicity to benthic organisms is determined by bioavailable metals concentrations in porewater. One ESB is based on the difference between simultaneously extracted metal (SEM) and acid volatile sulfide (AVS) concentrations in sediment (excess SEM). The excess SEM ESBs include a lower uncertainty bound, below which most samples (95%) are expected to be "non-toxic" (defined as a bioassay mortality rate ≤ 24%), and an upper uncertainty bound, above which most samples (95%) are expected to be "toxic" (defined as a mortality rate > 24%). Samples that fall between the upper and lower bounds are classified as "uncertain." Excess SEM ESBs can, in principle, be improved by normalizing for organic carbon (OC). OC is a binding phase that reduces metals bioavailability. OC normalization should improve the accuracy of bioavailable metal concentration estimates, thus tightening uncertainty bounds. We evaluated field-collected sediments from 13 studies with excess SEM, OC, and bioassay data (n=740). Use of the OC-normalized excess SEM benchmarks did not improve prediction accuracy. The ESB model predicts OC-normalized excess SEM exceeding the upper benchmark even when toxicity is not observed, because error in the OC normalization model increases at low OC concentrations. To minimize the likelihood of incorrectly identifying non-toxic samples as toxic, we recommend that OC normalization of excess SEM should not be considered for sediments with an OC concentration < 1% and is questionable for sediments with an OC concentration of 1-4%. Additional focused studies are needed to confirm or refine the minimum sediment OC concentrations that are applicable for reducing uncertainty in toxicity predictions due to excess SEM.

01.10 Fate and Effects of Metals: Mechanisms of Bioavailability and Toxicity

01.10.01 Conceptual Framework for the Ecological Risk Classification of Inorganic Substances

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The Chemicals Management Plan (CMP) is a Government of Canada initiative aimed at reducing the risks posed by chemicals to Canadians and their environment. Under the CMP, the Ecological Risk Classification of Organic substances (ERC) and the Ecological Risk Classification of Inorganic substances (ERC-I) approaches were developed to characterize the potential ecological hazard, exposure and risk of substances based on their properties in organisms and the environment. For organic substances, the ERC considers traditional empirical data (e.g., *in vivo* toxicity data), as well as new approach methods (e.g., *in silico*, *in chemico*, and *in vitro* data) in a weight of evidence framework. In contrast, inorganic substances are typically outside of the domain of applicability for most computational models and can cause interference in some *in vitro* assays. Therefore, the ERC-I approach primarily considers empirical hazard and exposure data to characterize the potential ecological risks of inorganic substances. A newly expanded ERC-I is currently under development to provide a fit-for-purpose risk assessment prioritization approach for inorganic substances. The approach leverages existing empirical hazard and exposure data and considers toxicity modifying factors and ambient background concentrations when these data are available. The incorporation of modelling approaches suitable for inorganic substances are currently being explored (e.g., Quantitative Ion Character-Activity Relationships). This presentation will outline the conceptual framework for a newly expanded ERC-I.

01.10.02 Understanding the Risk of Gadolinium in Aquatic Environments

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Gadolinium (Gd) is primarily used in contrast agents for medical imaging, with additional applications in metallurgy, neutron shielding, and other niche applications. Both Gd and Gd-based contrast agents (GBCAs, the chelated form for imaging) are not removed by standard wastewater treatment processing and therefore there is concern they are being released into surface waters and this may pose threats to the aquatic ecosystem. This study investigates the potential for environmental risks of different forms of Gd (such as free Gd and GBCAs) using the Grand River in southwestern Ontario as a model ecosystem. The Grand River watershed serves as an ideal location to investigate Gd and other rare earth elements (REEs) as it contains a variety of land uses, such as urban, industrial, and agricultural, as well as five MRI preforming facilities, that make use of GBCAs, and multiple waste-water treatment plants, potentially contributing to gadolinium or other REEs in the watershed. Total and dissolved Gd concentrations (along with other REEs) 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 show measurable levels of multiple REEs in the watershed. The potential for adverse effects of inorganic Gd and GBCAs were assessed using standard toxicity test methods with *Daphnia magna* and bioaccumulation studies with rainbow trout (*Oncorhynchus mykiss*). Initial results indicate that calcium, carbonates, and dissolved organic matter may act as toxicity modifying factors by impacting the bioavailability of Gd. Additional work is ongoing to determine if transmetalation reactions can occur when GBCAs are in the presence of other metals which could results in the displacement and release of free gadolinium in solution. The data generated in this study will help develop knowledge on the potential environmental impacts of Gd, GBCAs and REEs, and inform future research needs. This research is supported by a NSERC Strategic grant with funding from Environment and Climate Change Canada and additional contributions from Avalon Advanced Materials Inc. and GeoMega Inc.

01.10.03 Metal Speciation Alone Does Not Explain Metal Toxicity in the Rainbow Trout (Oncorhynchus mykiss) Gut Cell Line (RTgutGC)

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Bioavailability of metal complexes is poorly understood. The rainbow trout (*Oncorhynchus mykiss*) gut cell line (RTgutGC) was used to investigate bioavailability, bioreactivity and toxicity of different metal species of two non-essential (silver and cadmium) and essential (copper and zinc) metals. Visual Minteq, a chemical equilibrium model, was used to design different exposure media to allow formation of free ion, and charged and neutral metal complexes. Dose response curves were calculated using a multiple endpoint cytotoxicity assay measuring metabolic activity, cell membrane and lysosomal integrity. Bioavailability of different metal species was evaluated using ICP-MS and bioreactivity was measured by quantification of mRNA levels of metal response genes, such as metallothionein (MT), glutathione reductase (GR) and Zinc Transporter 1 (ZnT1). Speciation calculations showed metal species were dependent on anionic media composition in inorganic media. Cysteine avidly chelated all four metals reducing metal toxicity, bioavailability and bioreactivity. In the exposure media, silver and cadmium showed affinity for chloride, copper for phosphate, and zinc remained primarily in its free ionic form. Interestingly, free ion concentration in solution correlated significantly with effective concentrations 50 (EC_{50}) for the essential elements copper and zinc but not for the non-essential elements silver and cadmium. Silver and cadmium bioavailability was affected by chloride and bicarbonate concentration in solution, respectively. Moreover, calcium concentration was inversely correlated to toxicity and bioreactivity of cadmium and zinc. Non-essential metals silver and cadmium were more bio-reactive inducing a higher MT and GR response. This study shows that extracellular metal speciation alone is not sufficient to explain metal toxicity, and that other processes like co-transport with anions, metal bioavailability and bioreactivity are also important to explain metal toxicity.

01.10.04 Unexpected Modifications of Rare Metal Internalisation Kinetics (Ga, La, Pt) in the Presence of Natural Organic Matter (NOM) in a Freshwater Microalga

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In ecotoxicology, there is a growing interest for several rare and/or technologically critical metals including gallium, lanthanum and platinum. Their relatively recent and increasing use in anthropic activities (e.g. catalysts, new technologies and anti-cancer treatments) have led to an increase in their environmental mobility. As they reach aquatic systems, these metals can interact with organic ligands and especially Natural Organic Matter (NOM). The consequent formation of organic complexes would be expected to reduce metal uptake by living cells. Indeed, according to the Biotic Ligand Model (BLM), metal uptake can be expressed mainly as a function of free metal ion concentration when certain key assumptions are verified. This model is very robust and has been validated in many conditions, in laboratory experiments as well as in some field investigations. However, results from this work on rare metals internalisation kinetics (Ga, La, Pt) in *Chlamydomonas reinhardtii* appear to be in conflict with the BLM. For a constant total metal concentration, internalisation is sometimes significantly enhanced instead of reduced in the presence of NOM. Uptake modification seems to depend on NOM composition and vary greatly from one source to the other, as demonstrated with Suwannee river standards and NOMs from Ontario, Canada. Moreover, a significantly enhanced uptake in the presence of complex organic ligands raises the issue of the impact of these metals on microalgae in realistic environmental conditions (with ubiquitous NOM). It demonstrates the importance of investigating the role on NOM in metal interactions with aquatic organisms, primary producers being of great ecological importance.

01.10.05 Acute and Chronic Toxicity of Yttrium and the Influence of Toxicity Modifying Factors on Bioavailability and Toxicity of Yttrium to Daphnia magna

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Yttrium (Y) is an important technology critical element (TCE) being increasingly used in new technologies. The resulting growth in demand leading to exploration, mining, and refinement as well as e-waste generation and wastewater discharges raises concerns about its potential impacts on the aquatic ecosystems. Dissolved organic matter (DOM) modifies the biogeochemistry of these elements. Currently, there is little data on bioavailability and toxicity of TCEs and the toxicity modifying effects of DOM. This project investigates the toxicity and bioavailability of Y and the influence of toxicity modifying factors on toxicity and bioavailability of Y to *Daphnia magna*. Acute and chronic toxicity tests with and without added DOM as well as with increased Ca^{2+} and Na^{+} were performed following Environment Canada and USEPA standard test methods. The exposure solutions were prepared using a low hardness medium (0.25mM $CaCl_2$, 0.078mM $NaNO_3$, 0.078mM KCl, and 0.25mM $MgSO_4$, pH6.8). Different sources of DOM at a concentration of 10mg C/L, and high Ca^{2+} at 1.5mM and Na^{+} at 3mM were tested. The acute EC50 of Y to *D.magna* was 2.2mM and the chronic EC50 is 0.24mM for reproduction and 5.5mM for growth (wet weight). The addition of DOM and Ca^{2+} offered protection against Y toxicity to *D.magna*. Measured total and dissolved concentrations of Y in acute tests were similar but in chronic exposures, dissolved concentrations were lower than total due to the addition of food to the exposure medium. This study contributes data towards establishment of water quality guidelines for TCEs in freshwater. This research was funded by NSERC via a Strategic Grant with funding from Environment and Climate Change Canada and contributions by Avalon Advanced Metals Inc. and Resources GeoMega Inc.

01.10.06 Effect of Yttrium and Lanthanum Mixtures to Daphnia magna

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Yttrium (Y) and Lanthanum (La) are two rare earth metals frequently used in the high technology industry. Y and La occur together in a mineral ore called monazite. These elements can be reintroduced into the environment during mining and processing of mineral ores, manufacturing of high-tech devices, and/or disposal of said devices. There is a lack of knowledge of the aquatic effects of Y and La particularly as mixtures. The combination of the lack of knowledge and the still increasing levels in usage of these elements is a cause for concern regarding the potential for environmental effects. The objective of my study was to investigate the effects of Y and La alone and as mixtures using acute toxicity to *Daphnia magna* and a toxic unit approach. Standard 48-h single metal toxicity tests were conducted with *D. magna* neonates following Environment and Climate Change Canada methods to determine the EC_{50} for each individual metal. Mixture exposures were then designed based on converting the EC_{50} concentrations to toxic units (TU) and applying a 4x4 matrix isobologram approach with 0.15, 0.25, 0.5, 0.75 TU. The acute mixture test was that responses follow additivity and isoboles of 1.25, 1.0, 0.75, 0.5 and 0.25 TU were assessed. Tests were conducted in an artificial soft water medium with a hardness of 50 mg/L $CaCO_3$ and a pH of 6.8 at 21°C without the addition of food. The single metal toxicity tests resulted in an EC_{50} of 0.52 mg/L for La and 0.54 mg/L for Y (using measured dissolved concentrations). Acute mixture tests revealed an additive toxicity at low concentrations while at higher toxic unit combinations, synergistic effects were observed. Acute toxicity results were used to design targeted chronic tests. 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. and Resources GeoMegaA Inc.

01.10.07 Insight on How Dissolved Iron Can Modulate Copper Homeostasis in the Freshwater Alga Chlamydomonas reinhardtii

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Iron and copper are two essential elements for all living organisms. They are involved in several cellular functions and can sometimes interact to accomplish in concert several metabolic tasks. In our previous work, using *C. reinhardtii* as a test organism, we have shown that iron modulates copper uptake and toxicity. This result suggested that, under certain environmental conditions, iron would have a significant effect on copper homeostasis in algal cells. To investigate this hypothesis, various methodological approaches were used. Examination of copper intracellular distribution coupled with metallomic and proteomic approaches, have enabled us to understand that the concentration of iron in the water affects the intracellular fate of copper, in particular within cytosol (heat-stable proteins and peptides) and organelles. In addition, coupling the short-term uptake of copper and the expression of certain genes involved in iron and copper assimilation in *C. reinhardtii*, allowed us to highlight the fact that iron modulation of the sensitivity of algae to copper could be not only physiological in nature (feedback mechanism) but also attributable to protein complex formed with ferroxidase FOX1 and permease

FTR1. This study sheds new light on the importance of essential elements on the assimilation and internal management of toxic metals by aquatic organisms.

01.10.08 Lithium Accumulation by River Biofilms

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Lithium (Li) is a central element in the current global energy transition. As such, its use has more than doubled in the last decade, in particular for the manufacture of Li-Co batteries. However, its accumulation and toxicity towards aquatic organisms have been poorly studied. Biofilms are microorganism communities living in submerged substrata in aquatic ecosystems. As they are ubiquitous and relatively easy to sample, biofilms are of particular interest in environmental impact assessment. Moreover, they are known to accumulate metals as a function of their concentration and speciation in ambient water. This project aims at examining Li accumulation in river biofilms in order to assess their potential use as bioindicators of Li exposure in freshwater.To that end, biofilms were colonized in the *Gave de Pau* in the PERL outdoor open-stream mesocosms (TotalEnergies platform, Lacq, France) and were exposed to Li under controlled conditions of light and temperature in laboratory. The first experimental step of the study was to optimize a washing procedure to discriminate between total and intracellular accumulated Li. Various rinsing agents (Na, K, Mg and Ca) at different concentrations were tested on biofilms exposed to 1 mg/L Li for 24 h. Lithium accumulation was then examined in biofilms exposed to increasing concentrations of Li (0.01 mg/L to 100 mg/L) for 7 days. Lithium concentrations were measured by ICP-AES in both exposure media and acid-digested biofilms. Studied biofilms naturally contained 12 ± 3 µg of background Li per g of biofilm dry weight (µg/g_{dw}). That accumulation was found to increase to 25 ± 5 µg/g_{dw} in biofilms exposed to 0.94 ± 0.01 mg/L Li for 24 h. Intracellular Li accumulation was then determined after a washing step with K, which was identified as the best washing agent at a ratio [K]:[Li] = 10. Intracellular Li concentration was measured to be 14 ± 1 µg/g_{dw} and 18 ± 5 µg/g_{dw} in the control and exposed biofilms. That result suggests that Li was poorly accumulated upon Li exposure for 24 h. Exposure to 7 days confirmed that result with a 2-fold increase in intracellular Li while Li concentrations in exposure media increased 548 times. Overall, these results suggest that Li poorly accumulates in the studied biofilms, which is in agreement with its reported low toxicity on microalgae (EC_{50} in the mg/L range). Currents experiments aim to better understand the kinetics of Li accumulation and its controlling parameters.

01.10.09 Influence of Water Temperature on Copper Toxicity to Raphidocelis subcapitata

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The temperature, which influences the metabolic rate and physiologic process of living organisms, is a seasonal variation parameter expected to increase at least 2 °C because of global warming. Furthermore, there is a convergent agreement of many studies that high water temperatures will intensify eutrophication problems that threaten public health and degrade ecosystems. However, there is little information about the impact of temperature variation on environmental contaminant effects to aquatic organisms. To date, many studies have demonstrated that copper toxicity to microalgae species relies on water chemistry, but the influence of water temperature on copper toxicity has not been studied. This information is essential for a more accurate copper risk assessment in light of climate change. In this study, the influence of water temperature on copper toxicity to the growth of microalgae *Raphidocelis subcapitata* was investigated. Toxicity tests were conducted according to the standard methods, with temperatures varied from 18 to 30 °C. Controls (without copper) and metal solutions were prepared at least 24 hours before the algal test using the buffer 3-(N-morpholino) propanesulfonic acid (MOPS), and the final pH value was adjusted to 6 units using NaOH and HCl. The algal growth was monitored for 72 hours by measuring the fluorescence intensity at 435/685 nm of excitation/emission wavelength. The specific growth rate and algal growth inhibition were evaluated using the formula provided in the OECD guidelines, and the median effective concentration (EC_{50}) was estimated using the log-logistic dose-response curve. The result showed that temperature enhanced algal growth, which was in agreement with previous studies. Besides, copper became less toxic by increasing the water temperature from 18 to 24 °C; the EC_{50} increased from 23.3 ± 2.4 to 50.4 ± 3.7 µg-$Cu_{dissolved}$/L. However, the toxicity increased at 27 and 30 °C; there were no significant differences between these two conditions ($p \geq 0.01$). The low reproduction rate at low temperature and the high metabolism at high temperature might promote higher copper toxicity. Overall, the result suggests that the range of 21 to 24 °C testing temperature recommended in the standard guidelines (OECD-TG 201-2011) is the lesser protective condition against copper toxicity in light of global warming.

01.10.10 The Effects of Winter Cold on Acute and Chronic Cadmium Bioaccumulation and Toxicity in a Freshwater Fish

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Trace metals like cadmium (Cd) are important contaminants to freshwater fish in temperate regions. Fish also experience large seasonal temperature fluctuations that can potentially affect their exposure and sensitivity to metals, due the profound influence of temperature on biological and chemical processes. . However, temperatures effects are often overlooked in ecotoxicology studies, which are mainly conducted at ambient lab temperatures (i.e., 15-20°C). This knowledge gap is even more important at the low temperature extremes experienced during the winter. Our study investigates the effects of a winter cold temperatures on acute and chronic Cd bioaccumulation and toxicity in a freshwater fish: the banded killifish (*Fundulus diaphanus*). Killifish were gradually acclimated to either cold winter (4°C) or warm spring (14°C) water temperatures, then exposed to either no Cd, low Cd (0.5 µg/L) or high Cd (5 µg/L) sub-lethal concentrations . After 2, 5 and 28 days of Cd exposure, we measured Cd bioaccumulation within various tissues (gills, liver, gut, kidneys, and muscle) and are currently measuring markers of oxidative stress (e.g. protein carbonylation and catalase activity) in gills and livers. Cadmium was the most concentrated in the gills and liver, accumulation increased over time and was typically lower in cold-acclimated fish, likely due to slower Cd uptake rates. However, this lower Cd bioaccumulation may not translate into a proportionally lower toxic response in cold-acclimated fish, because of the additional stress they may experience from winter temperatures. Overall, our study aims at deepening our understanding of the influence of seasonal temperature on metal toxicity in aquatic organisms, in order to improve environmental risk assessments for metals.

01.10.11 Dietary Iron Supplementation is Linked to Short-Term Enhancement of Swimming Activity but Metal Dysregulation During Prolonged Treatment in Zebrafish (Danio rerio)

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Urbanization and increased urban runoffs have resulted in elevated levels of iron in many freshwater environments. The elevated availability of iron has been associated with metal loading into aquatic invertebrates and prey of fish. Under these circumstances, the iron content in the diets of fish may be increased by many thousand-fold. Additionally, the diet is considered the most important source of iron in fish. However, the implication of dietary iron remains unclear in fish and is mostly overshadowed by waterborne studies. To address these gaps, the present study examined the impact of dietary iron on key determinants of survival for fish, including swimming performance and respiratory metabolism. Adult zebrafish were exposed to 10 (low), 400 (control), and 2000 (high) mg iron/kg via the diet, for a duration of 20 or 40 days. Swimming performance was assessed using swim tunnel respirometers and the effects of dietary iron on critical swimming speed, oxygen consumption rates, metabolic parameters, and energy utilization were examined. Our results demonstrated that fish fed on a high iron diet exhibited an enhanced swim performance following 20 days of exposure. However, following prolonged iron supplementation at day 40, a significant elevation of iron is observed in the brain and intestine, suggesting dysregulation in iron homeostasis in these organs after chronic exposure. This reveals that iron supplementation at high concentrations can alter metabolic performance in the short term and disrupt iron balance following prolonged exposure which may have severe implications on the wellbeing of fish in metal-contaminated waters.

01.10.12 Salts Are Metals Too: Progress in Physiological Evaluation of the Multi-Ion Toxicity Model in Fish

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The Multi-Ion Toxicity (MIT) model is a theoretical framework based on electrochemistry, specifically the Goldman-Hodgkin-Katz equation and related formulations, for predicting the toxicity of various major salts to aquatic organisms. It posits that a certain depolarization of the transepithelial potential (TEP) at the gills will be associated with toxic effect. We will give an overview of several years of research aimed at physiological evaluation of the model and its assumptions. Specific topics will include (i) evaluation of immediate TEP responses with up to 10 salts in 5 species of freshwater fish (fathead minnow, channel catfish, bluegill, goldfish, rainbow trout), and the benefits of Michaelis-Menten curve-fitting to the data; (ii) evaluation of the roles of cations versus anions in the responses; (iii) evaluation of TEP responses and plasma ions in rainbow trout after 4 days of sublethal exposure to these salts; (iv) evaluation of the influence of background water hardness on immediate TEP responses to major salts in fathead minnow; (iv) evaluation of the interactive effects of two other major influences - dissolved organic carbon (DOC) and water pH – on immediate TEP responses to major salts in rainbow trout. Overall, our results provide strong support for the evolving MIT model and point to future research directions. Views expressed are those of the authors and do not necessarily reflect those of their affiliated organizations. (EPRI, Rio Tinto, NSERC).

01.10.13 Transepithelial Potential Responses to Sodium Chloride and Its Binary Mixtures After 4-Day Pre-Exposure to Major Salts in Rainbow Trout

B. Po, C.M. Wood, University of British Columbia / Zoology

The transepithelial potential (TEP) of freshwater aquatic organisms is being evaluated for its relationship to the toxicity of major salts in recent years, in support of the development of the Multi-Ion Toxicity (MIT) Model. Chronic exposure to moderate salt levels interspersed by acute exposures to higher levels may be a realistic scenario in nature. Since acclimation of fish to metals or salinity can alter their tolerance to these and other factors, it is important to study how pre-exposure to sublethal levels of major salts may affect their TEP responses. After a 4-day pre-exposure to sublethal concentrations (50% of LC50) of individual major salts (NaCl, Na_2SO_4, $NaHCO_3$, KCl, K_2SO_4, $KHCO_3$, $CaCl_2$, $CaSO_4$, $MgCl_2$, $MgSO_4$), the TEP responses of rainbow trout (*Oncorhynchus mykiss*) to a geometric concentration series of NaCl alone, and of NaCl in the presence of the pre-exposure level of the other salts, were compared. We found that for the NaCl alone series, the absolute TEP became more negative in the lower concentrations of NaCl when fish had been pre-exposed to any of the different types of salts when compared with non-acclimated fish. Most of the salt pre-exposures (except NaCl and KCl) also led to a minor positive TEP at lethal concentrations of NaCl. Therefore, for both reasons, the ΔTEP responses (relative to background) at higher NaCl concentrations were increased as a result of pre-exposure. For binary salt series, there was a steeper increase of TEP in the KCl/NaCl series, this could be related to the higher toxicity of NaCl when KCl was present. The significant change of the baseline TEP by the Ca and Mg salts had however masked the effect of NaCl on absolute TEP. These findings should be taken into account in further refinements of the MIT Model for the prediction of the toxicity of binary salt mixtures. (EPRI, NSERC).

01.10.14 The Unifying Principles of Transepithelial Potential Responses to Increased Salt Concentration of Fathead Minnow (Pimephales promelas) and the Water Flea (Daphnia magna)

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Freshwater ecosystem health is threatened by elevations in major ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}) from anthropogenic activities including road de-icing salts, fracking spills and mining. The increase in ambient ion concentration has detrimental effects on freshwater organisms, causing iono- and osmo-regulatory stress. Transepithelial potential (TEP) was measured in response to increased ambient Na, K, Ca and Mg salts in fathead minnow (FHM) (*Pimephales promelas*) and *Daphnia magna*. Using the Michaelis-Menten Model and previously published reference toxicity data, the two data sets were analyzed. The analysis revealed that there is a strong correlation between toxicity in FHM and toxicity in *Daphnia magna* across different salts. Significant positive relationships were observed between the inverse of affinity (K_m) and toxicity (Lethal Concentration 50 (LC50) values) for the various salts in both species. As such, a high affinity for ΔTEP response (low K_m) correlates with high toxicity (low LC50) for the same salt, and *vice versa*. A potency relationship has been identified and is similar between the two very different species, even though the absolute values may be quite different. The relationship shows that the potency of a salt in depolarizing the TEP is related to its potency in causing lethality. This result suggests that electrochemical modeling could be used to predict the short-term ΔTEP value at the gills, and in turn the associated toxicity in each species. Although the mechanisms of ionoregulation and sensitivity to toxicants varies greatly between fish and daphnids, we have noted significant unifying principles in the transepithelial potential (TEP) responses, an endpoint predictive of toxicity, to major ions. This finding will be important in the further development of predictive models such as the Electric Power Research Institute's (EPRI) Multi-Ion Toxicity Model, for regulating major ions and determining toxicity thresholds for freshwater ecosystems (EPRI, NSERC Discovery).

01.10.15 The Effects of Coal Ash Concentration on the Development, Reproduction, and Bioaccumulation of Trace Metals in Planorbella duryi

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Coal ash (CA) is produced in high quantities and contains various trace metals known to be toxic to both terrestrial and aquatic organisms. Following combustion, power stations can dispose of their treated CA waste in lined or unlined ponds or discharge them into nearby waterways, allowing contaminants to leach into the surrounding ecosystem. While previous studies have examined environmentally relevant concentrations of trace metals on aquatic vertebrates, few have assessed the effects of these coal ash leachates (CALs) on aquatic invertebrates. Thus, the goals of this study were to assess the impact of CALs on 1) the development of embryonic *Planorbella duryi*, 2) the reproduction of adult *P. duryi*, and 3) the bioaccumulation of trace metals within adult *P. duryi* tissues. To create CALs, 0g to 100g of CA obtained from a local repository was added to glass vessels containing 1L of synthetic water and adjusted to a pH of 7.0 using HCl. All CALs were vacuum filtered after settling for

48 hrs and aliquots analyzed for trace metals using ICP-OES. Embryonic *P. duryi* clusters (< 24hrs old) were exposed to each leachate for 10d and images were obtained every 24hrs to assess viability and development stage. For the reproductive assay, one adult *P. duryi* was placed into a glass vessel and exposed to a specific leachate treatment level for a 9d exposure period. After 9d, the exposed adult was paired with an unexposed individual in a separate glass vessel filled with synthetic water for 9d and newly-laid clutches quantified every 24hrs. For the bioaccumulation study, individual adults were exposed to a specific treatment for 9d, after which the footpad muscle, kidney, and digestive gland were dissected out, weighed, digested, and analyzed for trace metals using ICP-OES. Preliminary results indicate the lower treatments (1 g/L) exhibited accelerated development, whereas the higher treatments (50 g/L and 100 g/L) displayed delayed growth. While this study is currently ongoing, we expect 1) a dose dependent decrease in the number of viable embryos and egg clutches laid by the adults and 2) the bioaccumulation of trace metals within the digestive glands and kidneys of the adults above exposure concentrations. This study will provide essential information on the effects of CALs on the development, reproduction, and bioaccumulation of metals within an aquatic invertebrate and suggests possible effects on trophic level interactions within aquatic ecosystems.

01.10.16 Gastropod Shells As Bioindicators of Metal Contamination: Use of Imaging Techniques in Mass Spectrometry

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Gastropods are ubiquitous mollusks living in aquatic ecosystems. Their univalve shells made of calcium carbonate have the interesting feature to grow throughout the mollusk lives by integrating certain chemical elements present in their environment, including metals. Relationships have been established between the concentration of metals in their whole shells and in their ambient water, and this as a function of the exposure time. Gastropod shells might be a useful tool for biomonitoring and recording temporal changes in metal concentration in freshwater. However, the structure of shells is composed of several layers, each of which have different formation process, chemical composition and possibly affinity for metals. The aim of this study is thus to examine metal localization and speciation within gastropod shells in order to better understand their distribution and fate within shells. Two imaging analysis techniques are currently optimized to explore the elemental and molecular composition of shells: Laser Ablation coupled with Inductively Coupled Plasma Mass Spectrometry (LA-ICPMS) and Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS). Analyses were performed on cross sections of *radix* shells collected in the *Gave de Pau* in the PERL outdoor open-stream mesocosms (TotalEnergies platform, Lacq, France). LA-ICP-MS analyzes highlighted three kinds of elemental distribution: (i) homogeneous (Ca, Sr and Pd), (ii) preferentially located on the internal and external shell surface (Ba) and (iii) only located on the external part of the shell (Co, Cu, Ni, Pb and Zn). This latest distribution suggests that metals preferentially accumulate in the fine external layer (*i.e.*, periostracum), in the underlying prismatic layer or in both. Detected metals may also come from biofilms present on the surface and not removed by washing. TOF-SIMS analyzes confirmed that the surfaces of the shells were covered with fragments of polypeptides and phospholipids, which could correspond to the periostracum of the shells or partly to the biofilm. Our current experiments focused on identifying which compartment (s) mainly sequester metals on the external part of the shell and on defining its link with the bioavailable metal concentration in the ambient environment.

01.10.18 Longitudinal Impacts of Forest Management on Mercury Bioaccumulation and Trophic Transfer

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Forest harvesting can increase the mobilization of mercury (Hg) into headwater streams and its conversion to methylmercury (MeHg), the form that bioaccumulates and biomagnifies through the food web. Headwater streams are important sources of organic materials and nutrients for downstream systems, but it is unknown whether the impacts of forest management seen in these small systems accumulates or dissipates at sites further downstream. Herein, we collected water, seston, food sources (leaves, organic matter), five macroinvertebrate taxa and fish (slimy sculpin) at 6 sites representing different stream orders (1-5) within each of three river basins with different intensities of forest management (minimal, moderate, high; based on % harvested and replanted trees in past 10 years), and assessed their MeHg (or total Hg for fish) and relative trophic levels ($d^{15}N$). Differences in the longitudinal trends in MeHg or total Hg versus drainage area were found in the moderate and high management basins for some of these groups when compared to the pattern in the minimal basin, suggesting that forestry had either cumulative or dissipative effects on these measures. For example, total Hg in fish increased from upstream to downstream in the minimal basin but showed no spatial trends in the basins with moderate and high intensities of forestry. Trophic level ($d^{15}N$) was a significant predictor of log MeHg or total Hg (fish) across samples within all 18 sites, explaining between 73 and 94% of the variability, and data pooled across all sites within a river indicated a lower slope in the basin with moderate forest management (0.27 vs 0.32 and 0.34). The latter appears to be due to higher MeHg in the lower- but not higher- trophic levels in this system. Overall, results suggest cumulative or dissipative effects of forestry on Hg levels in abiotic or biotic samples across a spatial gradient and some effects on the trophic transfer of this contaminant through food webs.

01.10.19 Mercury Bioavailability and Subsequent Accumulation by Rhinichthys Atratulus, Eastern Blacknose Dace, Inhabiting a Historically Polluted Fluvial Ecosystem in CT, USA

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Heavy metal pollutants can actively cycle in ecosystems for decades and enter local food webs. Historical heavy metals, such as mercury (Hg), accumulate in aquatic organism's tissues via uptake from water and assimilation from ingested food. Our study asked whether historic Hg pollution in the Still River watershed, Danbury, CT continues to accumulate in a resident freshwater fish, the Eastern blacknose dace (*Rhinichthys atratulus*). Danbury, CT, USA, was once considered the hatting capital of the world. Millions of hats were processed from 1780 to the early 20th century with mercury nitrate [$Hg(NO_3)_2$]. The factories that were located on the riverbanks, directly released their byproducts into the Still River and into some of its tributaries. We measured concentrations of Hg in fish, fish prey (i.e., benthic macroinvertebrates), water and sediments from sites that were historically polluted (i.e., legacy sites) with those that were not historically polluted (i.e., reference sites). We hypothesized that Eastern blacknose dace from the legacy sites would have significantly higher Hg concentrations than those from the reference sites. In contrast to our hypothesis, fish from most reference sites had significantly higher Hg concentrations than fish from historically polluted sites. Environmental concentrations of Hg in the sediment, water column, and suspended particulates did not correlate with fish Hg concentrations. In addition, the correlation of Hg concentrations in fish and prey were not significant.

However, our benthic macroinvertebrate data was limited to only one collection period. We found that the vast majority of the legacy Hg from the hat-making industry in the Still River is buried in the deeper sediment layers, and only a small portion is actively cycling and is bioavailable to the food web. The elevated Hg in reference sites was surprising, suggesting other processes, that are currently unknown, may impact the efficiency of the transfer of Hg from abiotic compartments into living organisms. More research is required in the Still River watershed to understand the processes and mechanisms that drive Hg mobility and bioaccumulation.

01.10.20 Biokinetic Modeling of Mercury Accumulation in Fishes: A Case Study in an Eastern TN Stream

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Mercury (Hg) is a potent neurotoxicant that bioaccumulates in aquatic organisms and biomagnifies across food chains, causing widespread impacts. Hg-contaminated fish threaten human health, emphasizing the need to understand the dynamics of this bioaccumulation. Bioaccumulation can be described by models of varying complexity, from the simplified bioconcentration factor which describes the partitioning of contaminants between water and the organism, to more sophisticated models which take into consideration metal speciation, complexation, and/or bioavailability. Biokinetic models have been developed to understand and predict the body burden of metals in organisms depending on exposure and uptake route (e.g. Luoma and Rainbow 2005). However, these models have traditionally been applied in simplified lab settings over short time periods, where complicating factors such as diet composition and growth are not relevant and variability in forcing functions is minimized. In this talk, we will present our results using this model to understand and predict the patterns of Hg bioaccumulation in redbreast sunfish and rock bass over 10 years in East Fork Popular Creek (EFPC), a Hg-contaminated stream located on the Oak Ridge Reservation in East Tennessee, a federally owned property managed by the U.S. Department of Energy. These data span over a decade of sampling surveys across multiple locations along EFPC and include data from marked and recaptured fish, allowing for fine scale parameterization of the model. Inputs to the model simulations include dietary and waterborne concentrations of Hg. Successful parametrization of this model allows for the prediction of sunfish body burdens in EFPC moving forward, to be tested by continued biological monitoring. Further, explicit estimation of the relative contribution of food and waterborne Hg exposure to sunfish bioaccumulation allows for the testing of various management strategies to decrease fillet concentrations below advisory levels.

01.10.21 Evaluation of the Effects of Maternally Transferred Selenium on embryo-larval Development in Mountain Whitefish (Prosopium williamsoni)

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Selenium (Se) is an essential trace element involved in important physiological and metabolic functions for all vertebrate species. Fish require dietary Se to maintain normal physiological function, however, elevated concentrations have been shown to cause effects in developing embryos and larvae. As Se has a narrow margin between essentiality and toxicity in some species, there is concern surrounding the potential for adverse effects of elevated Se exposure caused by anthropogenic activities. Relatively few studies have assessed the direct implications from field caught fish from mine-influenced locations on early life stages. This study assessed the effects of egg Se concentrations derived via maternal transfer on early life stage survival, development and growth of a salmonid, the mountain whitefish (*Prosopium williamsoni*). Fish gametes were collected from streams in the Elk Valley region of British Columbia, Canada, proximate to coal mining activities with a range of selenium exposures. Eggs were fertilized and reared in the laboratory through the onset of endogenous feeding. Larvae were assessed for survival, length, and weight, as well as incidence of deformities (skeletal, craniofacial, finfold) and edema based on a graduated severity index (GSI). Eggs from a total of 66 females were assessed with egg Se concentrations ranging from 15.5 to 54.0 µg/g (dw). There was no evidence of adverse effects associated with exposure to selenium. In total, 10,099 mountain whitefish fry were assessed for deformities and, on average, rates of moderate/severe deformity (i.e., GSI >1) were 1.9% in swim-up fry across all females sampled. These data demonstrate that mountain whitefish are relatively insensitive to maternally transferred Se compared to other salmonids, with an adverse effect threshold (EC10) of >54.0 µg/g dw.

01.10.23 Relationship Between Arsenic in Surface Water and Fish Tissue in Idaho; Implications for Development of Water Quality Criteria

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In 2019, Idaho collected 45 paired surface water and fish tissue samples to derive a state-specific bioaccumulation factor (BAF) for inorganic arsenic (iAs) as part of the development of new human health water quality criteria (WQC). This collection effort was represents a one-of-a-kind study given the large number of samples and the geographic coverage of the sampling locations. No statistically significant relationships were found between total arsenic (tAs) in surface water and fish tissue, iAs in surface water and fish tissue, or iAs in fish tissue and tAs in surface water. Fish body weight was the only parameter with a statistically significant effect on iAs in fish tissue. Fish less than 100g had a range of iAs in tissue from 0.2 to 9.4 mg/kg, while the majority of fish weighing greater than 100g had iAs in tissue of ≤ 1 mg/kg. The exception were the samples from the bridgelip sucker, the only primarily herbivorous fish in the dataset. The concentration of iAs in bridgelip sucker tissue increased with body weight, while in upper trophic level fish (piscivores) and middle trophic level fish (insectivores), iAs tissue concentrations decreased with increasing body weight. The ratio of iAs to tAs in fish declined with increasing body weight among all fish combined and when middle and upper trophic level fish (insectivorous and piscivorous) were evaluated separately. In lower trophic level herbivorous fish, the bridgelip sucker, the ratio of iAs to tAs did not change with increasing body weight. These results are consistent with the iAs biodimunition and arsenic speciation trends observed in other field and laboratory studies reported in the literature. The decrease in iAs in fish tissue with increasing size and trophic level are likely the result of metabolic transformation of accumulated iAs to organic As species by organisms in each level of the aquatic foodweb. The findings suggest the traditional paradigm employing a BAF to derive WQC may not apply to iAs in Idaho waters.

01.10.24 Tissue Metal Concentrations and Antioxidant Enzyme Activity in Sharks

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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 various shark species including Atlantic sharpnose, 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. This study provides reference levels of metal contaminants in the muscle tissue of several shark species collected over the past three years and provides insight into oxidative stress defenses in these top-level carnivores.

01.10.25 Application of Michaelis-Menten Kinetics to REE Biouptake From Soil: PLANTS Vs DGTs

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The uptake and toxicity of metals often have been interpreted in the context of the Biotic Ligand Model (BLM). While BLM was successfully used in predicting metal bioavailability in aquatic environments, its applicability to the soil-to-plant interface remains to be demonstrated. Despite the recent interest in assessing the risks associated with REEs, data on their bioaccessibility and bioavailability in soils are still relatively scarce. Diffusive gradients in thin films (DGT) is an *in situ* technique that was used to measure Ce, Nd, and Eu availability during prolonged deployments in an REE amended black garden soil for comparison to REEs root accumulation by durum wheat (*Triticum durum* var. 'Kyle') and Tomato (*Solanum lycopersicum L.*). For Eu and Nd but not Ce, depletion of the DGT-adjacent labile pool was observed with prolonged exposures and required metal replenishment by mixing or replacing the soil. The replenishment experiment demonstrated that the heavier of the three REEs (Nd and Eu) had slower association kinetics than the lightest of the three REEs (Ce). Plant available REE estimated by both $CaCl_2$ and DGT methods were linearly correlated over a wide range in total soil REE concentration. Root accumulation of REEs for both species was comparable with accumulation by DGTs. Depending on the plant available metal in the soil as estimated by $CaCl_2$, accumulation of REEs by plant roots can be described by a Michaelis-Menten kinetic equation -maximum uptake rate Vmax and dissociation constant Km (corresponding to root affinity constant for the metal, K_{REE}=1/Km). The smaller the affinity constant value, the higher the affinity of a plant root for the REE. Michaelis-Menten curves for Eu and Nd did not result in affinity constants different from zero. Very similar K_{Ce} for DGT and plants suggest that both plant species (monocotyledon and dicotyledon) have similar Ce transporter that could be well simulated by DGT. The observed K_{Ce} for dissolved Ce in soils for tomato, durum wheat, and DGT (4.91, 4.93, and 4.77, respectively.) were lower than reported for REE free ion biouptake by freshwater algae and were more similar to those reported for divalent metals, such as Ca and Cu.

01.10.26 Some Like Bugs Better Than Worms: Development of Bioaccumulation Models Relevant to Wildlife That Eat Arthropods and Flying Insects

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For many terrestrial risk assessments, invertivorous species (e.g., robins and shrews) drive risks due to life history characteristics that maximize exposure, including preference for prey that tend to have high body burdens. Most invertivorous species eat a broad range of dietary items, including soft-bodied prey (e.g., earthworms), as well as hard-bodied prey (e.g., arthropods). It is challenging to collect enough terrestrial invertebrate tissue mass for chemical analysis, so bioaccumulation models become a critical aspect of estimating dietary exposure to invertivorous wildlife. Standard bioaccumulation models for invertebrates developed by Oak Ridge National Laboratory and USEPA are commonly used in Superfund risk assessments; however, these models are based on earthworms. Recognizing that uptake of contaminants into arthropods and flying insects is different than in earthworms, and that site-specific sampling may be challenging, literature-based bioaccumulation models for arthropods and flying insects are needed. This presentation describes the development of arthropod/flying insect bioaccumulation models for metals. A literature search was conducted and data gathered by the US Army Center for Health Promotion and Preventative Medicine (USACHPPM) was used. Regression models were prioritized over median bioaccumulation factors (BAFs) when the regression model was significant (slope $p < 0.05$), met minimum correlation requirements ($R^2 \geq 0.2$), and data size requirements ($n > 10$). A series of model validation procedures were used to confirm the reliability of the model-estimated linear relationships between biota and soil concentrations. The goal was to assess the sensitivity and variability of the model estimates. Three model validation methods were conducted, including: data splitting based on studies, data truncation of extremes, and bootstrap resampling. For terrestrial arthropods, metals with regression models that met acceptance criteria are arsenic, aluminum, cadmium, and lead, and for aerial insects, arsenic, cadmium, copper, lead, silver, and zinc. Median BAFs were developed for the rest of the metals.

01.10.27 Metals Distribution in Roadside Soils and Himalayan Blackberries in Victoria, BC, Canada

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The Himalayan blackberry (*Rubus Armeniacus*) is a very robust perennial invasive plant that is well established in the Pacific Northwest. The delicious berries from the plants, including those growing along roadsides and rights-of-way are frequently collected for human consumption. To assess the potential risk associated with exposure to metal contaminants, samples of soil, blackberry leaves, roots and berries were collected from 25 locations along high traffic corridors, medium traffic areas and peri-urban neighborhoods with minor traffic in Victoria, BC, Canada. Total metal concentrations in the soils, roots, leaves and berries were analyzed by x-ray fluorescence and inductively coupled plasma mass spectrometry followed by the determination of the biological accumulation coefficient (BAC), biological transfer coefficient (BTC) and biological concentration factor (BCF) for the plants. As Pb and Zn concentrations in soil samples collected from high traffic corridors were relatively elevated compared to lower traffic areas while other metals such as Ba, Cu, Cr, Fe and Ni did not show discernable differences among the locations. In fact, the mean concentrations of As and Pb in soils from the high traffic corridors exceeded the Canadian Council of Minsters of Environment soil quality guideline for residential/parkland use. The calculated BAC, BTC and BAF were very low suggesting minimal metal uptake by the blackberry plants from the soils, however As and Pb levels in the berries from the high traffic corridors were much higher compared to the low traffic areas. A follow-up investigation involving washed and unwashed berries is ongoing to ascertain if the relatively higher As and Pb concentrations in the berries from the high corridor areas are associated with particulate matter on the berries.

01.10.28 Modeling of Global Hg Emission Contribution to Hg Air Concentrations at Some Canadian Monitoring Sites

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Mercury (Hg) is a global pollutant and threat to human and environmental health. Its long-range atmospheric transport affects the regional or global distribution of airborne Hg as well as deposition to terrestrial and aquatic ecosystems. In this study, we used a modeling technique to simulate Hg long-range transport and emission contributions from sources around the world to some locations in Canada. The simulations were performed with the Stochastic Time-Inverted Lagrangian Transport (STILT) model and a Global mercury emission datasets covering 1970-2012. In the simulations, 15-days back trajectories and footprints along them were calculated for each hour. Hg emission contributions from sources all over the world to a measurement site can be obtained by multiplying the footprints and mercury emission fluxes. The model was run for different Hg measurement sites over many years and the global emission contributions to those locations were examined. Areas and emission sources that contributed significantly were identified. The evolution of contributions over years was also analyzed.

01.10.29 Yellow Road Marking Paint As an Environmental Source of Lead (Pb): A Case Study of Parking Lot Dust in Toronto, Canada

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Much progress has been made to eliminate the use of lead paint, including recent advances under the umbrella of the Global Alliance to Eliminate Lead Paint. In North America, the total lead content in household paints and surface coatings is restricted to 90 mg/kg. However, specialty coatings such as yellow road marking paint, which commonly contain lead chromate pigments, may exceed this limit. Given the potential for traffic markings to erode and release lead-bearing particles, the continued use of lead chromate pigments for marking purposes poses a potential health hazard. Research examining the possibility of pigment particle exposures via dust resuspension and inhalation processes is limited to date. To address this, this study aims to assess the importance of yellow road paint as a source of lead in road dust samples (n= 45) collected from publicly accessible outdoor and indoor parking sites in 2020/21 in downtown Toronto, Canada. Paint chips were also collected from select locations (n= 6). Samples were acid digested and the elemental concentrations determined using ICP-MS and ICP-OES. Laser diffraction particle size analysis was used to characterize the potential for dust resuspension and inhalation of bioaccessible, thoracic fractions (< 10 µm). The statistical program R was used to examine associations between lead concentrations and other elements such as chromium to inform the identification of source emissions. Overall, parking dust was determined to have a median lead concentration of 116 mg/kg. Lead levels were found to be highly variable, ranging from 19.1 mg/kg to 1282 mg/kg. Dust from indoor locations were found to be more enriched in lead compared to samples from outdoor sites (median: 154 mg/kg). Significant associations of lead with chromium indicated that yellow marking paint is the probable source for elevated levels of lead identified in indoor parking lots. This was supported by an analysis of yellow paint chips collected from certain locations, which had measured lead levels of up to 4.2 %. It was determined that an average of 10 % of particles in indoor parking dust samples were highly bioaccessible to the human lung (< 10 µm). For outdoor parking locations, an average of 3.9 % of particles were < 10 µm in diameter. Overall, the study results highlight that the continued use of lead chromate-based pigments in yellow paint for marking purposes, especially for indoor spaces, is a public health concern.

01.10.30 Intergenerational Effects of in Utero Arsenic Exposure

M. Colwell, N. Wanner, A. Rezabek, C. Faulk, University of Minnesota

Exposure to iAs during pregnancy disrupts normal DNA methylation patterns in developing offspring and leads to the onset of adult diseases such as type II diabetes (T2D) and obesity. More specifically, disruption during pregnancy attenuates the proper remodeling of the epigenome of the F1 developing offspring and potentially its F2 grand-offspring via disruption of fetal primordial germ cells (PGCs). However, there is a limited understanding between the correlation between the disease phenotype and methylation profile within the F1 offspring. Furthermore, we do not know whether iAs exposure alters epigenetic remodeling in F2 grand-offspring and if the F2 generation is also at risk for developing iAs associated diseases even without direct iAs exposure. Our study aims to understand the intergenerational effects of *in utero* iAs exposure on the epigenetic profile and onset of disease phenotypes within F1 and F2 adult offspring, despite the life-long absence of direct arsenic exposure within these generations. We exposed F0 dams to doses of iAs that represent the current WHO regulations (10 ppb), the average iAs exposure in developing countries (300 ppb), and half the known carcinogenic dose of chronic iAs exposure (2300 ppb) throughout pregnancy until the birth of F1 offspring. Our results demonstrate that iAs exposure targets both somatic cells and primordial germ cells, resulting in sex specific physiological changes in weight and glucose tolerance. This disease phenotypes of failure to thrive, T2D, and obesity are not characterized until late life in F1 and F2 offspring.

01.11 Immunotoxicology: Effects of Environmental Contaminants on the Immune System and Their Relevance to Risk Assessment

01.11.01 Transcriptomic Responses to Immune System Challenge in American Kestrel Nestlings Exposed to the Flame Retardant Isopropylated Triarylphosphate Esters (ITP)

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Isopropylated triarylphosphate esters (ITP) are components of several flame retardant mixtures, including Firemaster® 550 (FM550). ITPs are a complex mixture of ortho-, meta-, and para-substituted isomers that contain three aryl groups, each having at least one isopropyl group. These mixtures are known to cause various toxic effects in non-avian species, including neurological, developmental, and cardiac abnormalities. However, research on avian species, and particularly the immunotoxic effects of ITP, is still lacking. Although ITP levels have not been previously reported, other FM550 constituents have been detected in a range of habitats and wildlife across the globe, including in avian tissues and eggs. This potential toxicological threat combined with infectious disease outbreaks in avian species is cause for concern. In order to address some of the knowledge gaps, we exposed American kestrel (*Falco sparverius*) hatchlings to ITP and challenged them with polyinosinic : polycytidylic acid (Poly I:C), to simulate viral infection. Kestrels were gavaged daily starting at hatch with safflower oil or 1.5 ug/g bw/day of ITP suspended in safflower oil. All of the ITP hatchlings and a subset of the controls were injected on days 9 and 15 with Poly I:C. One subset of controls was sham injected with PBS. The nestlings were sacrificed on Day 22, necropsied, and spleens preserved in RNALater for transcriptome analysis by RNAseq. We observed stark differences in responses to both ITP and Poly I:C between male and female nestlings. Male and female birds differed in the number and types of altered immune-related pathways and gene ontology (GO) terms. In general, male birds had stronger transcriptomic responses to poly I:C and female birds had stronger transcriptomic responses to ITP. Only one immune pathway was enriched in ITP male nestlings relative to sham controls, 7 in ITP males relative to Poly I:C stimulated males and, 17 in Poly I:C stimulated males relative to sham controls. In females, 19 immune pathways were enriched in ITP birds relative to sham controls, 18 in ITP birds relative to Poly I:C stimulated females, and 4 in Poly I:C stimulated females relative to sham controls. Therefore, in males it appears that ITP exposure depressed the response to Poly I:C, whereas in females, ITP elicited a stronger response. The results of this study provide the first indications of immunomodulatory effects of ITP in birds.

01.11.02 Immune Endpoints and Plasma Perfluoroalkyl Substance Concentrations in Wild Smallmouth Bass

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Perfluoroalkyl substances (PFAS), also known as "forever chemicals", are a broad class of compounds with a variety of applications including aqueous film forming foam used for fire-fighting and training, a wide range of consumer products, and other industrial purposes. They have been widely detected in humans, wildlife, and the environment. Immunotoxicity and reproductive endocrine disruption have been linked to PFAS exposure in both humans and fish. We have recently detected PFAS in plasma samples taken from wild smallmouth bass (*Micropterus dolomieu*) during fish health assessments. Immune endpoints developed for wild smallmouth bass were also integrated into the health assessments in 2016-2019 to monitor and evaluate changes in immune status and look for signs of immunomodulation. Smallmouth bass in this region have faced a high prevalence of disease and mortalities, as well as reproductive endocrine disruption, with no single or consistent explanation. The challenge now is teasing out whether PFAS concentrations in the blood have any correlation with immune function endpoints. The health assessments took place in spring before spawning and fall during recrudescence at multiple sites in the Chesapeake Bay watershed. Assessments included recording gross abnormalities and collecting tissues for histopathology, gene expression, plasma hormone analysis, aging, and immune function for 20 adults at each site. The immune endpoints included bactericidal activity against *Yersinia ruckeri* and *Aeromonas veronii* bv. sobria, respiratory burst potential, and lymphocyte mitogenesis. Blood was also collected and plasma was archived. Select plasma samples were analyzed for PFAS. Preliminary immune function results reveal yearly, seasonal and site differences in immune function. At least four different PFAS compounds were also detected in every fish analyzed, with total plasma PFAS concentrations up to 644 ng/ml, but this varied among and within sites. Associations between immune endpoints and PFAS concentrations will be presented, recognizing the complexity and interactions of other factors such as disease status, and stressors associated with climatic factors, land-use, and a suite of environmental contaminants measured monthly in surface water.

01.11.03 The Marine Medaka Oryzias melastigma as a Model for Developmental Immunotoxicity

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In concert with the developmental origins of health and diseases (DOHaD) hypothesis, it is argued that environmental exposures of the more vulnerable developing and weaker immune system in embryos, newborns, and children may trigger immune pathologies. While critical windows have been defined for adaptive immune system development, knowledge about sensitive developmental periods of the innate immune system is still scarce in vertebrates. Here we propose the use of the immunotoxicological model organism marine medaka (*Oryzias melastigma*) to develop a molecular timeline of the innate immune system maturation. The gene expression of innate immune initiators (*C1q, MBL, TLR3, TLR5-membrane, TLR5-soluble*), mediators (*IL1ß, MYD88, M-CSF*), and effector (*LYZ*) revealed significant developmental changes between 9 and 11 days post-fertilization. To identify the susceptibility of these critical windows to environmental pollutants, perfluorooctenaesulfonate (PFOS) and its replacement, perfluorohexanesulphonic acid (PFHxS), will be employed as model compounds. Both are common environmental pollutants of current concern. PFOS is known to impair immune function during development and in adult organisms, while little is known about the immunotoxicity of PFHxS. PFOS and PFHxS exposures will be conducted with and without immune challenge using the fish pathogen *Edwardsiella tarda*. Impacts on the molecular (immune gene expression) and organism level (host resistance assay) will further validate the identified critical window. The generated data will substantiate and forward tools for future DOHaD research with a focus on critical windows during immune system development and developmental immunotoxicity. Expanding the marine medaka model for developmental immunotoxicology will accelerate the discovery of new immune markers and treatment targets to improve the risk assessment of per-and polyfluoroalkyl substances and their replacement compounds.

01.11.04 Interrogating Microglia As Critical Mediators of PFOS-Induced Neurotoxicity

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Per- and Polyfluoroalkyl Substances (PFAS) are a class of global toxicants that are highly resistant to environmental degradation. Exposure to perfluorooctane sulfonate (PFOS), a prevalent PFAS congener, dampens adaptive immune responses in children. However, it is not known whether PFOS exposure affects the development and function of microglia, the resident innate immune cells in the brain. Using a single cell image analysis pipeline, we found that PFOS exposure produced a rounded, activated microglia morphology in developing zebrafish. To assess microglial function following PFOS exposure, we performed a minor brain injury and examined the recruitment and persistence of microglia at the injury site. We found that PFOS-exposed embryos exhibited a heightened microglial response to brain injury and that the exacerbated response was not due to changes in inflammatory cytokine signaling or an increase in cell death. Therefore, we examined other factors in the microenvironment that may modulate microglial development and behavior. We used the photoconvertible calcium indicator CaMPARI to perform functional neuroimaging in zebrafish larvae and observed an increase in global as well as regional brain activity following PFOS exposure. The observed increase may reflect aberrant connectivity associated with the failure of microglia to refine neural networks. Alternatively, the increase in neuronal firing may drive the observed activated microglial phenotypes and alter microglial response to injury. Using optogenetics, we were able to induce a ramified, less activated state in microglia and rescue the exacerbated microglial response to brain injury in PFOS-exposed embryos. Furthermore, using qRT-PCR, we found that PFOS exposure caused a significant upregulation of *p2ry12*, a G-coupled protein receptor that is directly involved in microglia activation and migration toward ATP at injury sites. We are currently conducting experiments to determine if neural silencing is sufficient to rescue the altered microglial morphology in PFOS-exposed embryos and the microglial response to brain injury. In addition, we are examining whether other PFAS congeners alter neural network formation and/or the microglial development and function.

01.11.05 Comparing the Respiratory Burst In Vivo and In Vitro After Exposure to Per- and Polyfluoroalkyl Substances

D. Phelps, J.A. Yoder, North Carolina State University / Molecular Biomedical Sciences

The United States Environmental Protection Agency currently estimates that there are more than 9,000 per- and polyfluoroalkyl substances (PFASs), which are used to produce non-stick cookware, food contact materials, hydro- and oleophobic textiles, fire-fighting foams, and more. Due to their unique chemistry, they are ubiquitous, persistent, and mobile in the environment. This has made exposure to PFASs commonplace, and studies have estimated that 98% of Americans have detectable serum levels of multiple PFASs. These compounds have also been detected in remote wildlife, further illustrating their wide-reaching impact. Several studies have reported that these compounds are immunotoxic in humans and wildlife; 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 and an *in vitro* human neutrophil-like cell culture model to investigate innate immune function after exposure to environmentally relevant PFASs. Using non-teratogenic or non-cytotoxic concentrations, the respiratory burst was measured as a functional readout of innate immune function. Neutrophils, and other phagocytes, induce microbicidal reactive oxygen species through the respiratory burst to defend the host against pathogens. Preliminary data show that some PFASs are capable of inhibiting the respiratory burst *in vivo* and *in vitro*. While experiments are ongoing, potency, as measured by logistic regression, was similar between the two model systems, which may indicate evolutionary conservation between zebrafish and humans in their response to PFASs. Current studies are exploring this hypothesis, whether exposure to PFASs confers susceptibility to infectious disease, and what mechanisms may be responsible for this immunosuppressive phenotype.

01.11.06 How Do Perfluorinated Alkyl Substances (PFAS) Affect Macrophage Function?

A. Connors, North Carolina State University; J.A. Yoder, North Carolina State University / Molecular Biomedical Sciences

Immune function can be impaired by environmental contaminants. One class of chemicals recently shown to interfere with the immune systems is per- and polyfluoroalkyl substances (PFAS). Previous work has focused on impacts on the adaptive immune system, though disruptions to the innate immune system have also been identified. These studies have demonstrated the effects of PFAS on the numbers of innate immune cells, cellular signaling, and functional endpoints. For example, our lab has determined that certain PFAS can reduce neutrophil oxidative burst *in vitro* and in larval zebrafish (*Danio rerio*). To complement these neutrophil studies, we will run multiple assays to evaluate how macrophages are affected by exposure to several different PFAS. After assessing how PFAS exposure influences the viability of human macrophage-like THP-1 cells, we will evaluate how exposure affects macrophage phagocytosis. Macrophage populations derived from zebrafish and from THP-1 cells will be challenged with fluorescent heat-killed *E. coli* bioparticles. Phagocytic index and number will be measured with flow cytometry. Based on these functional assays, we will select 2-3 specific compounds to use in studies that will elucidate currently unknown molecular mechanisms of PFAS immunotoxicity. To this end, we plan to conduct transcriptomic analyses on PFAS-exposed macrophage-like THP-1 cells to identify differentially expressed gene networks. Understanding how PFAS impact 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.

01.11.07 Immunomodulation Associated With Perfloroether Acids, Understudied PFAS

J.C. DeWitt, T. Woodlief, E. Tobin, East Carolina University / Pharmacology and Toxicology

One sub-class of per- and polyfluoroalkyl substances (PFAS) that has emerged in drinking water sources in North Carolina (NC) is perfluoroalkyl ether acids (PFEAs). These have been identified in raw and finished drinking water and measured in blood of people consuming the water. Little toxicological data are available for these compounds. However, for other PFAS, immunotoxicity is an outcome of concern. We applied a suite of functional and observational immunotoxicological assays to evaluate understudied PFEAs using male and female C57BL/6 mice orally exposed for 30 days. Functional assays included T cell-dependent antibody responses (TDAR) and natural killer (NK) cell cytotoxicity. Observational measures included immunophenotype of splenic and thymic lymphocytes and lymphoid organ weights. We also included toxicological endpoints, liver weights and peroxisomal enzyme activity, linked to PFAS exposure. Four different PFEAs, including those with 3-, 4-, 5-, and 7-carbons, were evaluated: perfluoro-2-methoxyacetic acid (PFMOAA), perfluoro-3-methoxypropanoic acid (PFMOPrA), perfluoro-4-methoxybutanoic acid (PFMOBA), and Nafion byproduct 2 (NBP2). Doses for 3-, 4-, and 5-carbon compounds were 0.5, 5, and 50 mg/kg and doses for NBP2 were 0.5, 1, and 5 mg/kg. Relative potencies were: PFMOPrA = PFMOBA < PFMOAA < NBP2 for liver weight increases and TDAR decreases; PFMOPrA = PFMOBA = PFMOAA < NBP2 for peroxisomal enzyme activity, NK cell cytotoxicity, immunophenotype, and lymphoid organ weights. Changes induced by NPB2, a 7-carbon PFEA was not surprising. It is similar in structure to perfluorooctane sulfonic acid (PFOS), a legacy and immunotoxic PFAS, and was expected to induce immunotoxicity. What was notable about NBP2 is that it also was acutely toxic at 50 mg/kg, which is why it was administered at lower doses compared to the other PFEAs. Changes in liver weight and TDAR by PFMOAA were, however, unexpected. As a 3-carbon compound, it was expected to produce little to no immunotoxicity, as was observed with PFMOPrA and PFMOBA. Results for PFMOAA and NBP2 are particularly concerning in eastern NC as PFMOAA was present in the highest concentrations a drinking water source and while NBP2 was present in low concentrations in this river, it was detected in the blood of residents consuming the water. Our data demonstrate that commonly held assumptions about the contributions of carbon chain length to PFAS toxicity may not be applicable to all members of the class.

01.11.08 Immunotoxicological Convergence of Anthropogenic Toxicant Exposures and Coronavirus Disease 2019

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Anthropogenic environmental toxicants, including inhalational exposure to ambient airborne particulate matter (APM) or oral exposure to chemicals of emergent concern (e.g., polyfluoroalkyl substances or PFAS) can disrupt protective immune competence. Cells of the innate immune system, especially professional antigen-presenting dendritic cells (DC), serve as a crucial link when sensing exposure to "environmental danger signals," such as APM or PFAS, and adaptive T cell-mediated immunity. Exposure to environmental toxicants can induce immune dysfunction that leads to chronic disease, exacerbates pre-existing chronic airway diseases such as pro-allergic asthma, or induces states of relative immune suppression, wherein innate and adaptive host immune systems fail to protect adequately against community-acquired infections. Precise mechanisms remain poorly understood; however, we previously showed that particulate matter (PM) with aerodynamic diameters of less than 2.5 micrometers ($PM_{2.5}$) and ultrafine PM (UFP) of less than 0.1 micrometers skew pulmonary immunity to a T-helper type 2 lymphocyte (Th2)-mediated pro-allergic pattern of response, characterized by increased secretion of IL-13, IL-4, and IL-5 and decreased TNF-alpha and IFN-gamma. Immunological refocusing to a Th2-dominant response was accompanied by a state of relative and reversible immune-suppression of DC function accompanied by dampened membrane-associated expression of pattern-recognition Toll-like receptors (TLR)-2 and -4. Exposure to APM provides favorable conditions for community-acquired opportunistic viral and bacterial infections. Similarly, others have reported the immune-modulating effects of PFAS and a novel role for perfluorobutanoic acid (PFBA): a unique breakdown product of PFAS. Like APM and UFP, PFBA traffics to, and accumulates in the lung, thus potentially disrupting pulmonary immunity, immune surveillance, and defense against community-acquired viral infections such as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and its progression to coronavirus disease 2019 (COVID-19). Evidence-based research findings of skewed or disrupted innate immunity support an immunotoxicological mechanism in driving susceptibility to community-acquired infections. Collectively, these data highlight both the under-appreciated effect of increased or prolonged exposure to suspected environmental immunotoxicants that might subsequently exacerbate pre-existing chronic airways disease, and worsened outcomes from community-acquired viral infections such as influenza and COVID-19.

01.11.09 An Immune Cell-Based Assay for Detecting and Monitoring Bioactive Constituents Within Oil Sands Process-Affected Waters (OSPW)

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The Alberta oil sands region contains the largest oil sands (i.e., bitumen) reservoir worldwide. Bitumen (unrefined petroleum) is extracted by mixing caustic hot water with oil sands sediment, generating large volumes of oil sands process-affected water (OSPW) as a by-product. OSPW is a complex mixture of constituents with demonstrated toxic properties that is stored long-term in man-made reservoirs known as tailings ponds. Various OSPW remediation strategies have been investigated over the past decades, yet there is no current consensus on how to effectively reclaim tailings ponds in an economically feasible way. As tailings ponds must be eventually remediated into independently functioning ecosystems, contemporary monitoring of OSPW is needed to track compositional changes over a spatiotemporal scale. Recently, our lab has developed an immune cell-based *in vitro* assay system for monitoring and potentially identifying specific constituents of potential concern within these complex samples using a mammalian macrophage cell line. Found in all animals, large numbers of macrophages populate tissues exposed to the external environment (i.e., skin, lungs, and the digestive tract), which places them at increased risk of exposure to environmental toxicants. Therefore, macrophages represent an excellent model for development of an immune cell-based biosensor to evaluate the potential effects of contaminant exposures. Using mouse macrophages, we have shown that different OSPW samples produce distinct bioactive signatures that are unique to each OSPW tested. Specifically, when compared to control water samples (i.e., municipal tap water and a reference water obtained from the Athabasca River), OSPW-exposed macrophages displayed activation signatures with significant upregulation of several antimicrobial and proinflammatory biomarkers. This presentation further shows how macrophages can be used to generate unique bioactivity profiles for different OSPW samples that are sensitively influenced by both microbial communities and chemical constituents. These profiles are expected to help inform industrial and governmental decision makers on OSPW toxicity, as well as validate the efficacy of the macrophage biomonitoring system to respond to induced changes upon OSPW exposure.

01.11.10 Evaluation of the Immunomodulatory Effects of Ethinylestradiol by the Experimental Infection of Common Carp With Aeromonas salmonicida

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In order to evaluate the immunotoxicity of environmental contaminants, the chemical effects on susceptibility to pathogen have been tested using fish and their pathogens. Our group developed a test method using common carp (*Cyprinus carpio*) as the host and *Aeromonas salmonicida* as the pathogen, and confirmed the reproducibility of the tests. With further optimization, we finally fixed the experimental conditions of chemical exposure/pathogen infection to detect the chemically-induced alteration of susceptibility to infectious disease in fish. In the present study, we examined whether our fixed method could detect the immunomodulatory effects of ethinylestradiol (EE2), known as an immunotoxic agent. Four tanks with 24 sexually immature common carp (body weight approximately 5.2 g) in each were prepared. Two of them were exposed to 50 or 200 ng/L EE2 for the entire experimental period, and the rest of them were used as the unexposed groups. At 7 days after the exposure test started, fish in the EE2 exposed groups and in one of the unexposed groups were infected with *A. salmonicida* (1.1 x 10^6 CFU/mL). The water temperature was kept at 23 °C, and the fish were fed dry pellets twice a day. Fish mortality was recorded daily until 18-day postinfection, and dead fish were removed from the test tanks. Body weight of all the fish was recorded. The typical symptoms, bleeding at ventral area and dorsal, anal, or tail fins, were observed in the *A. salmonicida* infected groups. The incidence rates of the symptoms (42–67%) were not different among the infected groups, suggesting that EE2 does not cause the immunosuppressive effects against carp. However, mortality rate was lower in the 50 ng/L-EE2 group (8%), compared to those in other infected groups (33% or 38%), which could be the low-dose effect of hormesis. In the same experiment, reductions of body weight in carp exposed to EE2 were detected. Therefore, we further tested and found that EE2 exposure caused weight loss in carp regardless of infection. Since low mortality rate and weight loss were observed in fish exposed to 50 ng/L of EE2, it is necessary to examine the effects of EE2 on weight loss and immune system at lower concentrations. In a previous study, long-term exposure to low concentrations of EE2 (1.5 or 5.5 ng/L) reduced the severity of a parasitic disease in rainbow trout. In a future study, we will investigate whether the similar immunomodulatory effect can be detected in our experimental system.

01.11.11 Ingestion of McLR Leads to Dysregulated Metabolic and Immune Pathways in Female Zebrafish: Implications of MicroRNAs

J. Hansen, U.S. Geological Survey / Columbia Environmental Research Center; J.B. Greer, U.S. Geological Survey / Western Fisheries Research Center

Environmental stressors including temperature, pathogens and toxins can have severe effects on fish health. One example is the threatened survival of the endangered Lost River sucker of Upper Klamath Lake (OR). It is hypothesized that environmental factors contribute to juvenile mortality including exposure to the cyanobacterial toxin microcystin, resulting in the lack of recruitment of juveniles into older age classes. During an initial pilot study, we found that the hepatic transcriptome of juvenile Lost River suckers was significantly dysregulated 24 hours after McLR ingestion. As a follow up, female zebrafish were fed an McLR diet (15 ug/day) for seven days and on the eighth day, fish that had received the McLR diet were placed back onto a standard diet to address aspects of recovery. In addition, fish were either injected with PBS or microbial pathogen associated molecular patterns (PAMPs) on the eighth day to assess potential immunomodulation due to prior McLR consumption. Control and experimental fish were sampled on days 3, 7, 10 and 13 where it was found that protein phosphatases (PP1) and associated regulatory subunits (e.g., PPP1CB) were implicated in the dysregulation of metabolic pathways and oxidative stress at each time point. We also found that expression of genes involved in antigen presentation (MHC, proteasome subunits) and inflammation (IL1B, IL8) post-PAMP stimulation were altered in response to prior McLR ingestion. Finally, several miRNAs were predicted to be upstream regulators controlling the hepatic, mRNA transcriptome response to McLR ingestion and immune stimulation. We have now characterized the hepatic miRNA transcriptome by miRNAseq that identifies potential miRNAs involved in silencing genes associated with metabolic processes and immunity. Taken together, this study provides additional insight about the ability of McLR to differentially modulate genes and pathways that may contribute to the health status of juvenile Lost River suckers of Upper Klamath Lake.

01.11.12 Comparative Response of Smallmouth Bass (Micropterus dolomieu) and Largemouth Bass (Micropterus salmoides) to a Short-Term Poly I: C Immune Challenge

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Disease outbreaks, skin lesions, fish kill events, and reproductive abnormalities have been observed in wild populations of Centrarchids in the Chesapeake Bay watershed (CBW). These adverse effects occur more predominantly in smallmouth bass (SMB) populations than largemouth bass (LMB) populations, suggesting a potential species difference in sensitivity to stressors that impact the immune response. To date there have been no comparative studies to identify differences in immune response between these two species. Our objective was to conduct an investigation on the physiological and molecular mechanisms of antiviral immune response in SMB compared to LMB. Polyinosinic:polycytidylic acid (poly I:C) was administered *in vivo* to juvenile SMB and LMB to mimic a viral infection and induce an antiviral immune response. Thirty fish per species were intraperitoneally injected with 10 µl g^{-1} fish of poly I:C or sterile phosphate-buffered saline (PBS), and this was repeated for three replicates per treatment and species. Ten fish were sampled at each time point of 24 h, 48 h, or 72 h post-injection to evaluate gene expression changes in a suite of immune related genes over time. At 72 h post-injection, blood smears were also collected for differential blood cell analysis. Our results indicated a shift in leukocyte profile in the poly I:C challenged groups in both species to a higher neutrophil : lymphocyte ratio compared to the PBS control groups, indicating a stress response was induced. The comparison also suggests a trend toward greater magnitude of the immune response in LMB relative to SMB. To our knowledge this is the first study to compare the antiviral immune response of smallmouth and largemouth bass.

01.12 Lab and Field Collected Invertebrates in Ecotoxicology: Challenges and Opportunities

01.12.01 How Well Do Laboratory Estimates of Exposure Predict Field Results?

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Laboratory experimentation is often used to evaluate factors affecting bioavailability, but these studies are rarely compared to field applications. In 2019 a pilot study was conducted on the effectiveness of field application of activated carbon to reduce the bioavailability of DDT and its metabolites. This provided a unique opportunity to run laboratory and field applications simultaneously. Four grid areas were randomly selected, and soil was sampled prior to carbon application. The toxicity and bioaccumulation of DDT and its metabolites were evaluated in *Eisenia fetida* prior to the carbon addition. Carbon was manually applied to the grid areas at a rate of approximately 2%. Simultaneously, a subsample of soil was maintained in the laboratory and 2% by weight of the carbon amendment was added. At three- and nine-months post carbon addition the toxicity and bioaccumulation of DDT and its metabolites were re-evaluated in *Eisenia fetida* in both the laboratory amended soil and soil collected from the field. These values were then compared to bioaccumulation in field-collected earthworms. The laboratory-amended soil had a greater reduction in bioavailability compared to field amended soils, with wild earthworms having intermediate results. This could be due to a variety of factors including differences in actual initial carbon amounts or loss of carbon in the field in addition to other factors. By increasing the understanding of the relationship between field and laboratory experiments, we can better understand the value of laboratory assays and their ability to extrapolate to field observations.

01.12.03 A Diversity and Functional Approach to Evaluate the Macroinvertebrate Responses to Multiple Stressors in a Small Subtropical Austral River

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Aquatic macroinvertebrates are considered effective bioindicators of ecosystem health due to their different sensitivities to habitat alteration and pollution. Macroinvertebrate community structures can be assessed using commonly used diversity-based approaches, however, functional trait-based approaches are increasingly introduced into bioassessments. The Hex River is subjected to intensive mining activities, while urban and industrial effluents, as well as treated and untreated sewage also contributes to pollution in the river. The present study aimed to assess macroinvertebrate responses to different stressors, determine whether diversity-based or trait-based approaches provide a better indication of responses and, to evaluate if metal bioaccumulation patterns reflect responses to this multi-stressor environment. The mining impacts, in combination with urban, industrial and sewage effluent, altered the macroinvertebrate community structure of the Hex River. The diversity-based approach indicated a clear difference between reference and impacted conditions but did not clearly differentiate between different stressors, while the trait-based approach distinguished the urban, industrial and sewage effluent sites from mining impacted sites. The use of both approaches are therefore recommended as they complement each other. From the present study, it is evident that macroinvertebrates can be considered as both reliable accumulation-, and response bioindicators to pollution from mining, as well as urban and industrial activities.

01.12.04 Iron Presence in Stream Restoration Projects in the Chesapeake Bay Region: A Mesocosm and Field Investigation

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Stream restoration projects are an essential step to mitigate negative effects associated with urban stream syndrome, such as flash flooding, increased nutrient loading, and disturbed hydrology in lotic habitats. Maryland has undertaken stream restoration projects to reduce sediment loading and nutrient transport to the Chesapeake Bay through use of Regenerative Streamwater Conveyance (RSC). Some restoration projects following RSC design have resulted in the presence of iron flocculate, which is likely caused by conditions favorable for denitrification through reducing conditions. Iron flocculate is not only aesthetically displeasing—it may also impact the overall health of benthic macroinvertebrate communities. Typically, iron exists in aquatic environments in its ferrous (dissolved/bioavailable/aqueous) or ferric (flocculate/particulate/solid) form. High concentrations of ferrous and/or ferric iron can be deleterious to lotic ecosystems. Mesocosms frequently serve as empirical linkage between controlled laboratory studies and field research; here we evaluated the effects of iron to a stream macroinvertebrate community under acidic and basic conditions to manipulate iron state for 60 days and conducted a short-term caged field experiment. Macroinvertebrates, representing multiple functional feeding groups were obtained through field sampling and a commercial laboratory for the mesocosm experiment. A total of 36 mesocosms were used for the following treatments, acidic (pH 5.5-7)+iron (0.6 mg/L); basic (pH7.5-9)+iron (0.6 mg/L) and the respective pH treatments without iron amendments. Three mesocosms from each treatment were terminated on days 21, 42, and 60. Optimal duration for the mesocosm exposures was 42-days, significant impact of the mesocosm environment was noticeable at 60-days. Iron flocculate (basic+iron treatment) resulted reduced amphipod survival at 0.6 mg Fe/L which is significantly lower than the chronic screening level value of 100 mg Fe/L. The field experiment evaluated effects of iron to macroinvertebrates by collecting caddisflies from a reference stream and placing them in enclosures within streams along an iron gradient. Preliminary results indicate that the experimental method was effective and that survival of caddisflies was generally lower in streams with high Fe. Collectively, these data suggest that iron in precipitate form may have more of an impact to macroinvertebrates compared to iron in the dissolved form.

01.12.07 The Influence of Caging on the Dreissenid Mussel Metabolome

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NMR metabolomics has been successfully applied to examine changes in the metabolome of dreissenid mussels with exposure to different pollutant scenarios in the environment. This advancement is promising for mussel biomonitoring studies as it provides in-depth information on the organism's response to chemical stressors. For deployment at sites of interest mussels are often caged to enable ease of collection at appropriate time points. Caged mussels can be transplanted from control sites to polluted sites, providing a controlled response for acute exposures. The same population can be sampled multiple times throughout the study period, controlling for reproductive status and age of the mussels. In a previous study, we observed a metabolic difference between *in situ* vs caged mussels at a single location. Here, we systematically evaluate the effect of caging on the mussel metabolome to determine if this handling technique might confound metabolomic comparative studies. *In situ* dreissenid mussels at two different sites (LMMB-04 and LMMB-05) in the Milwaukee Harbor, USA were placed in cages in June 2018. Four weeks later (July 2018), both caged and *in situ* mussels were collected from the two sites. Mussel metabolomes were examined using NMR spectroscopy with both sites showing subtle metabolic effects of caging. However, when examining both the sampling site and the handling technique, the sampling site accounted for the distinction in the data set. The metabolic effects caused by caging mussels were less influential than the environment of the site, thus verifying that the method of caging can be utilized on mussels for biomonitoring metabolomics in areas of interest.

01.12.08 Comparison Between the Sensitivities of Long-Term Laboratory-Reared and Field Populations of Ceriodaphnia dubia

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Ceriodaphnia dubia is an EPA-recommended organism for use in toxicity testing in certified ecotoxicology research facilities. It is a suitable test organism because of its simplicity to culture and responsiveness to toxicants allowing facilities to determine the potential impacts of contaminants in aquatic environments with high reproducibility. For testing convenience, *C. dubia* is often cultured for extended periods in the lab with little knowledge of how it impacts the organism's sensitivity and health. Long-term laboratory rearing of *C. dubia* cultures could impact how they respond to stressors and decrease the accuracy of test results calculated in the lab. This work investigated if a population of *C. dubia* cultured for an extended period in the lab was representative of three native field populations by systematically comparing their sensitivity to an organic and inorganic toxicant. The impacts of land use on the sensitivities of the three field populations were also explored. Comparative chronic toxicity tests with sodium chloride and the commonly used organophosphate insecticide, chlorpyrifos, were completed with the long-term laboratory-reared culture and three field populations of *C. dubia*. Preliminary results with the laboratory culture indicate that they are more sensitive to chlorpyrifos than previous studies. These results suggest that it could potentially benefit ecotoxicology research facilities to periodically introduce new individuals into lab cultures from wild populations to more realistically reflect contaminant responses of native *C. dubia*.

01.12.09 What Is the Significance of Reduced Mussel Growth Measured During a Bioassay?

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What is the significance of reduced mussel growth measured during a bioassay used in risk assessment? Although a few field studies have linked reduction in growth of fish with decreases in fecundity, whether growth endpoints from a bioassay of juvenile mussels can be used to predict effects of a toxicant on reproduction or populations in the field, is unknown. This uncertainty is compounded when considering the numerous life stages, at which effects on survival, growth, or reproduction might occur. We aimed to quantify the relationship between reduced mussel growth in a 28-day zinc (Zn) exposure and the subsequent effects on their long-term viability including survival and growth in good water quality and food conditions. To do this, we conducted a laboratory chronic bioassay with 6-week-old juvenile fatmucket (*Lampsilis siliquoidea*) in a 28-day zinc exposure (120 and 240 µg Zn/L) with endpoints of survival and growth (length and dry weight) following standard ASTM methods, and then transferred surviving mussels from the control and treatment groups into a culture pond and monitored their survival and growth for 12 weeks. The mean length and dry weight of mussels in the two treatments were significantly less than those from the controls at the end of the 28-day Zn exposure. Mussels from the control and the two treatments grew significantly in the culture pond during the 12 week grow-out period. Mussels exposed to Zn appeared to grow at the same rate as mussels in the control treatment, but they did not catch up to control mussels and were around 2-4 mm shorter in length and approximately half the dry weight of control mussels. Future studies will focus on longer Zn exposures (e.g., 90 days) and pond culture (up to 2 years), coupling to bioenergetic and growth models to understand the potential linkages of mussel growth to mussel populations and ecosystem services.

01.12.10 Transcriptional Biomarkers in Freshwater Mussels (Anodonta anatina)

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As stationary filter-feeders, bivalves risk exposure to dissolved and particulate pollutants present in the surrounding environment. Consequently, they are adequate study organisms for various research questions in ecotoxicology. For instance, the zebra mussel (*Dreissena polymorpha*) is occasionally proposed as a freshwater model organism, but this invasive species has little ecological relevance to many natural ecosystems. In contrast, freshwater mussels of the *Anodonta* genus are naturally distributed across Europe, Asia and North America. Duck mussels (*A. anatina*) occur abundantly in many European rivers and lakes. A complex life cycle makes laboratory culturing difficult, but field collected *Anodonta* show promise as study organisms in both laboratory and *in situ* studies. In a series of laboratory experiments, we therefore used *A. anatina* to assess a biomarker panel of transcriptional responses to pollutant exposure: catalase (*cat*), glutathione-S-transferase (*gst*), heat shock proteins 70 (*hsp70*) and 90 (*hsp90*), metallothionein (*mt*) and superoxide dismutase (*sod*). Adult mussels were acutely (96 h) exposed to copper or industrial wastewater, after which we assessed biomarker responses in gills and digestive glands, using reverse transcription quantitative polymerase chain reaction. Gill markers generally demonstrated monotonic responses to Cu exposure, in contrast to few pronounced responses in digestive glands. Considering observed background variation and a wide range of exposures (0.13 – 1 600 µg Cu/l), changes in response magnitude were relatively small. Specifically, modeled response fold-changes were

01.12.11 Evaluation of the Toxic Effects of the Dichlorvos Pesticide on Seven Specie of Cladocerans

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The D.D.V.P (DICHLORVOS: 2,2-Dichloroethenyl dimethyl phosphate.) is an organophosphorus pesticide considered highly toxic by the USEPA. Concentrations this pesticide from 0.05 at 10.12 mg/L have been detected in aquatic systems after its application in sanitary campaigns for livestock (sanitary bathroom). Because there are few studies of the effects of DDVP in aquatic organisms The objective of this study was to evaluate the toxicity of Dichlorvos in seven species of cladocerans: *Daphnia magna*, *Daphnia exilis*, *Daphnia pulex* , *Ceriodaphnia dubia*, *Moina macrocopa*, *Chydorus* sp. and *Simocephalus mixtus*. In addition their sublethal effects were evaluated by means of assessment of four biomarkers (growth rate, O:N index, lipoperoxidation and inhibition of acetylcholinesterase enzyme). Acute bioassays were performed, the organisms were exposed to 6 pesticide concentrations to determine the LC50. Subsequently tests with duration of 21 days were made where the organisms were exposed to two sublethal concentration (LC1 and LC10), for assessment of 4 biomarkers (growth rate, O:N index, lipid peroxidation and inhibition of acetylcholinesterase enzyme). The LC50 values obtained in the bioassays varied from 0.32 to 0.021 mig/l In the tests it was evident that the cladoceran *Daphnia exilis* was more sensitive to DDVP compared to other species. The O:N index had values below 9 fact indicates that organisms were in a high degree of stress. Growth rates of intoxicated organisms were between 19 to 49% lower than those observed in the control group. The average concentrations of Tbars registered organisms exposed to the pesticide show a direct dose-response relationship, since when increasing the time of exposure to DDVP increased the degree of lipid peroxidation in the tissues. A decrease in AChE activity was observed between 22 and 45% . The results of this study indicate that the effects of the pesticide DDVP in organisms under study are likely irreversible.

01.12.12 From Genes to Populations: Intraspecific Genetic Variation in the Sub-Lethal Effects of Harmful Algae

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Using invertebrate models in ecotoxicology to predict population level responses is central to understanding how sub-lethal exposure to toxicants translates to altering communities and ecosystems. Evaluating the magnitude of intraspecific variation in response to toxicants is a key step towards both better understanding how natural populations may respond to stressors and to identifying the genetic basis for variation in responses. To this end, we quantified the effects of a toxigenic strain of *Microcystis aeruginosa* on the survival, somatic growth rate, and reproductive output of 20 genetically distinct clones of *Daphnia magna* across three treatments (*Chlorella vulgaris* only, 2:1 *Chlorella*: *Microcystis*, and 1:1 *Chlorella*: *Microcystis*). We found considerable intraspecific genetic variation, including trade-offs in performance, at different concentrations of *M. aeruginosa*. Results were then plugged into population dynamic models for simulating communities with varying amounts of genetic diversity. We found a positive but non-linear relationship between clonal diversity and predicted population growth. Finally, we used whole genome sequences from each of our 20 clones to uncover associations between population genetic variables and response to *M. aeruginosa*. Taken together, this study reinforces the profound importance of intraspecific genetic variation in response to toxic insults and provides a pathway for linking toxicological responses from genes to populations.

01.12.13 Evaluation of Acute and Chronic Responses of Ceriodaphnia dubia, Pimephales promelas, Raphidocelis subcapitata, and Lemna gibba to Pulp and Paper Mill Effluents

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The US Environmental Protection Agency (EPA) currently has toxicity testing methods for effluent and ambient testing that were promulgated under the Clean Water Act Part 136. These include chronic and acute tests using several species; most commonly Ceriodaphnia dubia, Pimephales promelas, and, to a lesser extent, Raphidocelis subcapitata (formerly Selenastrum capricornutum and Pseudokirchneriella subcapitata). EPA's Office of Research and Development (ORD) has begun to focus on additional species or test methods to add to the suite of freshwater test species to provide additional options for evaluating toxicity. These include a shorter-duration chronic test method using Daphnia magna that may be a suitable alternative to the chronic test using C. dubia. Additionally, EPA may explore WET methods for an additional plant species (Lemna sp.). We evaluated the response of C. dubia, D. magna, R. subcapitata and L. gibba to exposure to treated pulp and paper mill effluent from seven mills during WET bioassays. This paper describes acute and chronic responses (growth, reproduction) in the context effluent chemistry, and provides insight into the relative effects of pulp and paper mill effluent exposure.

01.12.14 Effects of Imidacloprid on the Survival and Reproduction of Folsomia quadrioculata in Soil Microcosms

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Imidacloprid is one of the most commonly used neonicotinoid insecticides, used around the world for the controlling of insect pests. Despite being banned in the European Union, it is still used in other continents by the agriculture and forestry industries. Neonicotinoids contaminate ecosystems after their use and negatively affect non-target arthropods. One group commonly used to assess the impacts of neonicotinoids are the Collembola (springtails), due to their important ecological function, abundance, and exposure through diffusion of water from the soil via the cuticle. However, one problem faced by ecotoxicological studies is the challenge in trying to harmonise results and methodological practices between field and laboratory studies. This study assesses the effects of imidacloprid on Folsomia quadrioculata, an abundant species of springtail found widely distributed throughout a variety of habitats in the Northern Hemisphere. We used a population of F. quadrioculata sampled from an agricultural field margin in Ås, Eastern Norway. Soil microcosms were prepared from intact field-collected soil cores in the summer of 2019 and spiked to 0 mg kg-1, 0.004 mg kg-1, 0.02 mg kg-1, 0.1 mg kg-1 and 0.5 mg kg-1 imidacloprid per kg dry soil. The soil cores were defaunated by freezing and 20 adult individuals of F. quadrioculata were added to each microcosm, and kept at 15°C and constant humidity. Experiments were terminated at 4 different time points: 28, 35, 42, and 84 days, to assess the effects on adult and juvenile abundances through time, with the latter two timepoints chosen to align with a previous field study. At termination, animals were extracted and fixed, and the total numbers of adults and juveniles counted to assess the effects of imidacloprid on survival and juvenile recruitment. The number of individuals in control groups is expected to increase with time due to reproduction. However, within each time point, there was a reduction in the total number of individuals, adults, and juveniles at the highest exposure concentrations, 0.1 mg kg-1 and 0.5 mg kg-1, as compared with the control. The findings suggest that sublethal imidacloprid concentrations may lead to population decline by reducing juvenile recruitment and adult survival. After final analysis of results, this study aims to assess the viability of this methodology in combining the precision afforded by laboratory studies with the more ecologically relevant conditions of field studies.

01.12.16 Assessing the Ecotoxicity of PFAS (Per- and Polyfluoroalkyl Substances) to House Crickets (Acheta domesticus) Via a Novel Model System

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Per- and polyfluoroalkyl substances (PFAS) are environmentally persistent chemicals that have been widely used since the 1940s. They have been commonly used in aqueous film-forming foams (AFFF) for fire suppression and training on military installations. Their widespread usage has led to soil, groundwater and surface water contamination. Importantly, critical uncertainties exist regarding the ecotoxicity of PFAS. Although toxicity studies have been conducted on aquatic, avian and terrestrial vertebrates for common PFAS such as perfluorooctane sulfonate (PFOS), little is known about the effects on terrestrial invertebrates, which play a critical role in ecosystems. The goal of this research was to assess PFAS toxicity using a novel model test method on terrestrial invertebrates. 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, specifically for reptilian and avian species. Crickets were first exposed via a screening study to determine the most relevant PFAS substances. Perfluorooctane sulfonate (PFOS) and Perfluorohexane sulfonic acid (PFHxS) showed 100% mortality and 30-50% mortality at 30mg PFOS/L and 30mg PFHxS/L, respectively. Both PFOS and PFHxS are environmentally relevant chemicals, as they are the most commonly measured PFAS in surface water and soil for DoD installation with a history of AFFF use. Multiple studies assessing the toxicity of PFOS and PFHxS were then conducted. Crickets were exposed to 0, 0.003, 0.03, 0.3 and 3 mg PFOS/L via drinking water for 120 hours using a novel delivery method called the "Falcon Dress". The study was repeated multiple times to determine consistent results. For all studies we have seen consistent mortality of 30-50% at 3 mg PFOS/L. Crickets were exposed to 0, 1.5, 3, 10 and 30 mg PFHxS/L via drinking water for 120 hours using the same delivery method. For all studies we have seen consistent mortalities of 20 - 40% at 3 mg PFHxS/L and 60 - 70% at 30 mg PFHxS/L. Hence, this testing system has yielded consistent toxicity estimates for water-borne PFOS exposure to a representative terrestrial insect model. Both PFOS and PFHxS have demonstrated toxicity in terrestrial invertebrates, specifically house crickets (Acheta domesticus). This novel testing system and current results will improve our understanding of PFAS toxicity and risk to terrestrial ecological receptors.

01.12.17 Exploring the Role of Exposure Route (Diet Vs Drinking Water) on the Toxicity of PFAS to a Model Terrestrial Invertebrate, Acheta domesticus

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Per- and polyfluroalkyl substances (PFAS) are persistent industrial chemicals used in a variety of applications for decades. PFAS have been key ingredients in Aqueous Film Forming Foam which has been used to quickly extinguish fires and has seen widespread historical use, particularly related to aircraft. One PFAS, perfluorooctane sulfonate (PFOS), has been measured in humans, wildlife, and environmental media pointing to the importance of this (and other) PFAS as a globally relevant contaminant. Despite widespread occurrence, there is still considerable uncertainty regarding the toxicity of PFAS, including PFOS, on ecological receptors. For example, there are few data on the effects of PFOS on terrestrial invertebrates. Previously, the Aqplied Ecology and Ecotoxicology Lab at Towson University has developed a toxicity testing system for terrestrial invertebrates in which crickets (Acheta domesticus) were exposed to PFAS via drinking water. This testing system has provided robust and repeatable toxicity data for PFOS and PFHxS. Here, we developed methods to expose crickets to PFAS via the diet as a means of improving our understanding of how exposure route contributes to toxicity. Although still in development, we generate solid, laboratory food source for crickets that was then amended with PFOS. We included five treatment groups with food made from different concentrations of PFOS (0.003, 0.03, 0.3, 3, and 30 mg/L) and one control (no amended PFOS). There were four replicates per treatment with five crickets per replicate. An environmental control was also included in which no crickets were added to the tank but the food sources was present to account for changes in food weight due to water loss. Although were was some substantive water loss to the food, crickets were observed readily eating. Preliminary results showed 100% mortality at the highest PFOS treatment within 48 hours suggesting high toxicity to PFOS via the dietary route; these results are comparable to toxicity via drinking water exposures although additional analyses are needed to confirm this. Although methodological improvements are required, we anticipate that the results of these experiments can guide future work on PFAS and provide additional information related to toxicity of PFAS to terrestrial invertebrates. These data and insights may translate to improvements in ecological risk assessment of PFAS.

01.12.18 Effect of Temperature on Insecticide Toxicity to Multiple Life History Traits of a Soil-Dwelling Collembola: Implications for Populations in a Changing Climate

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Natural and anthropogenic stressors co-occur in nature, often causing antagonistic, additive, or synergistic effects on the performance of exposed organisms, as reflected in life history traits. Temperature, a strong natural driver of adaptations of organisms to their natural habitat, can attain stressful values. Accordingly, the adverse effect of a chemical, e.g., an insecticide, on organisms can be larger at stressful temperatures than at optimal temperatures, when other counteracting processes are either weak or absent. Imidacloprid is a globally used neonicotinoid insecticide, which persists in soil for >100 d at levels that can elicit adverse biological responses. Imidacloprid exposure has adverse effects on ecologically important non-target soil organisms playing important roles in litter decomposition and nutrient cycling, such as Collembola (springtails). *Folsomia quadrioculata* is a collembolan species abundant in many habitat types throughout the northern hemisphere. The thermal performance of this species has been characterized in detail previously, and the sublethal dietary exposure effects have been studied. Here, we have studied the modulating effects of optimal to heat-stress inducing temperatures (10, 15, 20, and 25°C) on the exposure to two continuous dietary sublethal imidacloprid concentrations (and a control) in *F. quadrioculata* hatchlings for an equal physiological duration (1200 day-degrees). We recorded multiple endpoints, e.g., body length, age at first reproduction, egg production, and survival. At 1200 day-degrees, imidacloprid had the strongest effect on egg production among all the endpoints studied. The largest difference relative to the control was observed at 20°C, whereas no eggs were found in any of the treatments at 25°C, which is consistent with the thermal adaptation of the focal species. Body length showed a stronger decline relative to the control at 25°C than at the other temperatures. In contrast, age at first reproduction and survival were not significantly affected by imidacloprid. The reduced egg production implies that long-term exposure to low imidacloprid concentrations could negatively affect the population size over time. Moreover, the temperature effects suggest these adverse imidacloprid effects can be aggravated by increases in soil temperature due to global climate change.

01.12.19 Monitoring Global Oxylipid Production in Caenorhabditis elegans Is a Preferred Method for Assessing Oxidative Stress

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Balanced levels of reactive oxygen species are key in critical physiological processes like redox signaling. However, excessive oxidative stress has been linked to a variety of toxicological endpoints, including cardiovascular disease, neurological disease, carcinogenicity, diabetes, and rheumatoid arthritis. The human enzyme cytochrome P450 2E1 (CYP2E1) metabolizes xenobiotics, sometimes producing more reactive species. Additionally, toxicant metabolism in the mitochondria may result in mitochondrial dysfunction, leading to further oxidative stress. *Caenorhabditis elegans* strains modified to target human CYP2E1 to the mitochondria or endoplasmic reticulum were exposed to styrene, a widespread pollutant. The resulting oxidative stress was measured using three traditional methods (protein oxidation, antioxidant protein expression, and lipid peroxidation) alongside measuring global oxylipid production. Oxylipids are potent signaling molecules involved in numerous physiological processes, including an organism's response to oxidative stress. These lipids were measured by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), in a cutting edge metabolomic approach to assessing oxidative stress. While the traditional methods showed either no variation or slightly increased oxidative stress in styrene-exposed worms, the LC-MS/MS method should prove to be more sensitive, with changes in several lipid classes, including isoprostanes and prostaglandins. Measuring changes in oxylipids will also help determine the mechanistic links between styrene exposure and oxidative stress, and relate lipid signaling pathways to established oxidative stress pathways. Additionally, the results of this study will contribute to the development of a novel high-throughput assay for assessing the systemic oxidative stress of emerging pollutants.

01.13 New Approach Methodologies (NAMs) to Evaluate Endocrine Pathways – Test Development Through Regulation

01.13.01 USEPA Endocrine Disruptor Screening Program (EDSP) Update on Implementation of NAMs

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The USEPA established the Endocrine Disruptor Screening Program (EDSP) in 1998 to screen certain substances for an effect in humans or wildlife that disrupts the estrogen, androgen, or thyroid pathways. The EDSP was designed to utilize a two-tiered screening and testing strategy where the Tier 1 battery, comprised of 5 in vitro and 6 in vivo assays, screen chemicals for the potential to interact with the endocrine system and Tier 2 tests identify adverse effects and establish dose-response. At the time, New Approach Methods (NAMs) including high-throughput, non-animal toxicological methods were not validated for use in a regulatory setting. There is now a growing desire to utilize NAMs in place of animal testing and, where possible, implement the 3Rs (reduce, refine, replace) to accelerate the pace of chemical safety risk assessments. The EPA published an EDSP21 workplan in 2011 which laid out a strategy to "pivot" the Tier 1 screening battery from using low-throughput or in vivo assays to high-throughput (HTP) assays and computational models. Since that workplan, the EPA has requested feedback on a variety of HTP assays and models for use in the EDSP from scientific peer-review panels in 2013, 2014 and 2017. In 2015 the EPA published a federal register notice further committing to the development and use of alterative NAMs to replace some Tier 1 assays. Some of the HTP and computational tools which have been reviewed are the ToxCast estrogen receptor (ER) assay set and pathway model, androgen receptor (AR) assay set and pathway model and the HTP H295R steroidogenesis assay. Other NAM products being developed and/or considered by the EDSP include the use of QSAR models, the SeqAPASS software program, bioactivity exposure ratios (BERs) and in vitro to in vivo extrapolation (IVIVE). The EDSP committed to establishing a transparent, scientifically sound, and regulatory implementable approach to using NAMs for the mandatory screening of pesticides. This approach will consider the strength and uncertainties of the NAM-tools in combination with the existing, validated assays in the EDSP tiered-framework and other scientifically relevant information as part of the approach to EDSP screening of pesticides. This presentation will summarize the scientific progress for the use and implementation of NAMs in screening and prioritizing chemical substances in the EDSP. *This abstract does not necessarily represent USEPA policy.*

01.13.02 Toward a Battery of Endpoints for Assessing Thyroid Hormone System Disruption in Fish

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Thyroid hormone system disruption (THSD) has been recognized as an import focus area in the context of hazard and risk assessment of chemicals where further development of assays and endpoints is needed. In particular for fish test guidelines no THSD endpoints are generally accepted yet. Various projects are currently being carried out to address this challenge, including the H2020 ERGO project, the EU DG Environment funded iFEDT project, the development of an adverse outcome pathway (AOP) network for THSD in fish (project 1.35 of the OECD AOP Development Programme), and the inclusion of THSD endpoints in fish test guidelines (project 2.64 of the OECD Work Plan for the Test Guidelines Programme). All these activities are focused on increasing our fundamental understanding of TSHD, reviewing existing knowledge, and developing a fish TSDH AOP network to support the selection of assays for inclusion in test guidelines. From the perspective of NAM development it is especially important to systematically document the high degree of life-stage specificity of the different THSD mechanisms and effects in fish. This level of resolution in the domain of applicability description is required to determine which endpoints are relevant in the context of non-protected life stage tests such as the Fish Embryo Acute Toxicity test (OECD TG 236), and which endpoints need to be assessed in juvenile or adult tests. A number of endpoints are currently well developed, including swim bladder inflation, eye development, thyroid follicle histology and thyroid hormone level measurements. Other endpoints under consideration include fin development, skin pigmentation and gene transcription in target organs. It is likely however that the level to which these individual endpoints are specific to THSD may differ. It is therefore anticipated that a combination of endpoints may be required in some scenarios. Overall, a sufficiently informative set of endpoints can likely be added to embryo-based tests to result in a significant reduction of animal testing. For those endpoints that need to be assessed in later life stages, further reduction in the use of animals can possibly be achieved by combining and extending existing test guidelines. One example of such an approach is the iFEDT project, which aims to combine the Fish Short-Term Reproduction Assay (OECD TG 229) and the Fish Sexual Development Test (OECD TG 234), and to add the assessment of THSD endpoints to the resulting test protocol.

01.13.03 Assessment of Endocrine-Active Contaminants in Surface Water Collected in the Great Lake Tributaries In Vivo and Vitro

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To assess hazardous chemicals in the environment, we have primarily focused on model species. However, vulnerable populations in the wild are not zebrafish or medaka. The impact of contaminants of emerging concern (CECs) should be evaluated on aquatic organisms in the Great Lake Tributaries. It is often not feasible to directly assess the impacts of CECs on these species due to the natural history of the species with long life spans (i.e., the Lake sturgeon) or because their conservation status precludes disturbances that would be associated with direct study (i.e., many freshwater mussel species). Therefore, this project's objective is to develop and assess the long-term effects of CECs in a multi-species in vitro that allow for the assessment of differences in species sensitivity to CECs without the need for organismal approaches. This approach is reliable, especially in endangered or threatened species.Utilizing a long-read transcriptomic analysis, we have identified 14 and 3 nuclear hormone receptors in hepatic RNA of the Lake sturgeon and hepatopancreatic RNA of freshwater mussels, respectively. In the hepatic RNA of the Lake sturgeon, we identified nuclear receptors for estrogen, androgen, thyroid hormone, vitamin-D3, peroxisome proliferator-activated, and others. Lake Sturgeon significantly expressed estrogen receptor 1 (esrl) in gonad and the inferior lobe of the brain, androgen receptor (ar) in the gonad, and thyroid hormone receptor-β (thrb) in the cerebrum, eye, Inferior lobe, and olfactory bulb. We reproduce these expression profiles in a trans-activation assay in vitro so that we can reconstruct tissue-specific responses of nuclear hormone receptors. Utilizing these assays, we have asses the estrogenicity of the surface water collected at the Great Lake tributaries in 2016-2019 in vitro. The results showed a significant difference among the ESR subtypes and species such as fathead minnow, bluegill sunfish, and largemouth bass. In vitro receptor activation assay via Bluegill sunfish estrogen receptor 1 showed relatively high sensitivity among the species and receptors. Concentrations of contaminants in the surface water were associated with the biological response of fathead minnow in 21-days expose in several endpoints. Therefore, we need further investigations into a diversity of receptors and species for the risk and hazardous assessments using local wildlife in vivo and vitro.

01.13.04 Evaluating Multiple Machine Learning Models for Endocrine Disruption Predictions

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Endocrine disruption is a major focus of toxicology research across industries, therefore human estrogen and androgen receptors are key targets of interest to understand if a molecule interacts with them. Downstream effects of receptor activation are difficult to anticipate without *in vitro* and *in vivo* testing, so the Environmental Protection Agency (EPA) has prioritized alternative approaches to evaluate endocrine bioactivity, including mathematical and computational methods. The EPA has used high-throughput ToxCast/Tox21 screening data of relevant targets and bioprocesses to calculate area-under-the-curve scores and predict the likelihood of chemicals to mediate endocrine activity. However, these ToxCast pathway models required a suite of *in vitro*assay data to generate a prediction. In contrast, machine learning (ML) methods are capable of prospective prediction from molecular structure alone and we have recently published on the broad applicability of ML to drug discovery and toxicology research. The current study summarizes some of this work which describes the application of several ML algorithms (including deep learning, Bayesian, and regression algorithms available to us) to the *in vitro* ToxCast/Tox21 data to generate suites of models to anticipate endocrine disruption. Model performance was evaluated by internal five-fold cross-validation metrics as well as external predictions with androgenic and estrogenic reference chemicals; these predictions were compared to results reported by the EPA's published studies. Furthermore, we have similarly modelled aromatase inhibition data, another component of in endocrine disruption. This study builds upon our previous work with Bayesian models that demonstrated a similar level of accuracy seen in previoUSEPA publications and expands techniques employed. We can also now add a comparison of the reference chemicals and their overlap with the model training sets using autoencoders to map the chemical space which would provide additional insights into the applicability domain of these models. In addition, we now have 15 different ML algorithms for providing further options for model comparisons. In summary, our results continue to demonstrate the utility of ML for endocrine activity prediction to fill data gaps. Combining the aromatase inhibition ML models with ER and AR model suites allows for more holistic assessment of endocrine disrupting potential to ultimately create an adverse outcome pathway.

01.13.05 Bridging the Gap: Integrating Systematic Review Strategies and New Approach Methodologies for the Cross-Species Extrapolation of Endocrine Targets

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The US Environmental Protection Agency's Endocrine Disruptor Screening Program (EDSP) is tasked with assessing chemicals for their potential to perturb the estrogen, androgen, and thyroid endocrine pathways. Traditionally, chemical screening for endocrine activity is performed using a tiered toxicity testing strategy that includes resource intensive in vivo studies. The EDSP has been exploring the use of new approach methods (NAMs) to prioritize and test chemicals more rapidly. However, most NAMs rely on a few model species which may not be sufficient to evaluate the broad diversity of species potentially impacted by chemical exposures. A NAM that can be used to predict biological pathway conservation across taxa and therefore extrapolate from model species to untested species, is the USEPA Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) tool. The SeqAPASS tool rapidly evaluates protein target sequence and structural similarity to understand conservation of biological targets. As with many NAMs, SeqAPASS is designed to help evaluate chemical-biological interactions at the molecular level. Science-based approaches for endocrine hazard and risk characterization, require the linking of molecular mechanisms to adverse apical responses. To link molecular endpoints captured through NAMs and traditional apical outcomes, tools and techniques are emerging that focus on the systematic identification, review, and evaluation of existing data. The integration of systematic review methods has maximized the use of existing toxicity data, reduced testing resources, and has strengthened transparency and confidence in risk assessments. In this study, SeqAPASS was used to assess the conservation of endocrine targets and generate molecular-level predictions of cross-species chemical susceptibility. Further, the latest tools in data curation science for systematic review practices were applied to anchor SeqAPASS-derived molecular-based predictions of species susceptibility with existing in vitro and in vivo data providing weight of evidence for the extrapolation of endocrine responses across species. Overall, this presentation describes a framework for understanding pathway conservation for endocrine targets across species and highlights both the need, and a strategy to bridge the gap between existing NAMs and current systematic review practices. The contents of this abstract neither constitute nor necessarily reflect USEPA policy.

01.13.06 Deciphering the Mode of Action of Thyroid Active Chemicals Using the OECD TG 248 - XETA (Xenopus Eleutheroembryonic Thyroid Assay)

G.F. Lemkine, Watchfrog S.A.

The XETA (Xenopus Eleutheroembryonic Thyroid Assay) is an *in vivo* assay using xenopus embryos to detect thyroid active compounds. Eleutheroembryo defines early life stages post-hatch which still depend on maternally deposited energy reserves. The association of embryo-larval developmental stages of fish or amphibians with the use of genetic markers is a highly advantageous ethical alternative. The test relies on transgenic embryos bearing a genetic construct where Green Fluorescent Protein expression is controlled by the thyroid pathway. Thyroid active molecules, hormones and endocrine disrupters induce increases or decreases in fluorescence, depending on their mode of action. The XETA was published as the OECD test guideline n°248 in spring 2019.

Inherently, the XETA provides a degree of information about the likely mode of action of thyroid active chemicals. Each chemical is tested either in the presence of Triiodothyronine (T3 spiked mode) or absence of T3 (unspiked mode), providing the opportunity to hypothesize on the mode of action. Thyroid receptor agonists induce an increase in fluorescence in the spiked and unspiked mode, while thyroid receptor antagonists induce a decrease in fluorescence in T3 spiked mode. Substances modulating the clearance or metabolism of the hormones modify the fluorescence only in the presence of thyroid hormones. Increases or decreases in fluorescence are observed depending on the enzyme inhibited by the substance. We are developing strategies using additional co-treatments to help distinguish between the different modes of action of the substances modulating the fluorescence. By adding additional co-treatments such as thyroid receptor antagonist (i.e. the pharmacological inhibitor NH3), it is possible to delineate whether the effect is mediated through the thyroid receptor and could be excluded as a potential false positive. Another example is that by comparing the results obtained with either a T_4 or a T_3 co-treatment, substances acting as deiodinase inhibitors can be identified. Depending on the deiodinase inhibited, the change in fluorescence of T_4 and T_3 can potentially be observed in both directions. We are validating this co-treatment strategy on a set of reference compounds to support the addition of these co-treatments in the XETA test guideline (OCED 248).

01.13.09 Verification of In Vivo Estrogenic ACTivity for Four Polyfluoroalkyl Substances (PFAS) Identified As Estrogen Receptor Agonists in High-Throughput Screening

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Per- and polyfluoroalkyl substances (PFAS) are a large class of fluorinated organic chemicals of concern due to their broad occurrence and persistence in humans and the environment and potential health effects. In response to these concerns, over 140 PFAS were screened for 81 different transcription factor activities in two multi-factorial transactivation assays. Over 40 distinct PFAS structures including perfluorooctanoic acid (PFOA) and many less studied PFAS showed activity against the estrogen receptor (ER). Most were partial agonists with maximum efficacy less than that of 17β-estradiol (E2), with 1H,1H,8H,8H-perfluoro-3,6-dioxaoctane-1,8-diol (PFDOD) identified as a notable exception that displayed full agonist activity. The aim of the present study was to evaluate whether the ER agonist activity detected through *in vitro* high-throughput screening would translate into estrogen-dependent effects in fish *in vivo*. Adult male fathead minnows (*Pimephales promelas*) were exposed to four ER-active PFAS including PFOA, PFDOD, 1H,1H,8H,8H-Perfluorooctane-1,8-diol (FC8-diol) and 1H,1H,10H,10H-Perfluorodecane-1,10-diol (FC10-diol) for 4 days. Five concentrations, with the maximum concentration set at either 50x (FC8-diol, FC10-diol) or 5x (PFDOD and PFOA) the 50% activity concentration (AC50) of the *in vitro* assays were tested. Following exposure, liver tissues were collected and expression of four genes known to be modulated by estrogen exposure - *vtg* and *esr1*, upregulated; *apoeb* and *igf1*, downregulated – was evaluated by quantitative real time polymerase chain reaction. Data collected to date show that while PFOA did not elicit impacts on gene expression consistent with those observed for the positive control (E2), 0.15-1.5 mg FC8-diol/L caused induction of *vtg* and *esr1* expression and suppression of *igf1* and *apoeb* at magnitudes similar to that elicited by the E2 positive control. These data suggest that at least some novel PFAS are able to elicit effects *in vivo* consistent with exposure to a steroidal estrogen. These results suggest ER-active PFAS likely warrant further monitoring in the environment to evaluate whether environmental exposures to these compounds may reach concentrations sufficient to elicit adverse effects previously associated with estrogenic endocrine disruption. *The contents of this presentation neither constitute, nor necessarily reflect, USEPA policy.*

01.13.12 AOP Development; Aromatase Inhibition and Androgen Receptor Agonism Lead to Male Biased Sex Ratio Via Impacts on Gonad Differentiation

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In teleost fish, the balance between steroidal estrogens and androgens is essential during sexual differentiation and this balance is in turn dependent on the availability and activity of steroid synthesizing enzymes such as aromatase, the enzyme responsible for the conversion of androgens to estrogens. Given the important role that steroid hormone signaling plays in sexual differentiation, it is not surprising that exposure to some types of endocrine active substances during critical periods of development can result in skewed phenotypic sex ratios. The present study utilized the adverse outcome pathway (AOP) framework to organize available evidence linking aromatase inhibition and androgen receptor agonism to male-biased sex ratios in fish. A literature search was conducted using 'aromatase inhibition', 'male biased sex ratios' and 'androgen sex reversal' in combination with 'fish' as the main keywords. Publications describing exposures or experimental treatments of teleost fish during the critical period of sexual differentiation and including both gonadal histology and an indicator of final population sex ratio were included as relevant. Additionally, a novel mathematical model linking male biased sex ratio to projected alterations in population trajectories was developed. Collectively, there was moderate to high scientific support for the causal linkages between aromatase inhibition or androgen receptor agonism and male biased sex ratios in fish. These two AOPs, focused on impacts during the period of sexual differentiation (i.e., early life stages), complement a broader network of AOPs documenting potential hazards that endocrine active chemicals pose to fish, thereby further supporting predictive approaches to hazard and risk assessment. *The contents of this abstract neither constitute, nor necessarily reflect, official USEPA policy.*

01.14 New Approach Methodologies – From Research to Risk Assessment

01.14.02 Data Gathering Under TSCA and Consideration of New Approach Methods

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The Toxic Substance Control Act (TSCA) requires EPA to consider alternative data with the goal of reducing vertebrate testing (Section 4(h)). Prior to requesting testing in vertebrates, the statute requires that EPA consider reasonably available toxicity information, computational toxicology and bioinformatics, and high-throughput screening and related prediction models. EPA also considers data from chemical groups and read-across from analogues, when implementing Section 4(h). While many new approach methods (NAMs) exist which could be considered in place of vertebrate testing, EPA's Office of Pollution Prevention and Toxics (OPPT), which administers TSCA, publishes a list of acceptable NAMs with regular review that includes predictive models used historically in the program's risk assessment process as well as internationally accepted (non-vertebrate) test guidelines. This presentation discusses how NAMs are considered within a framework developed for environmental hazard data gap/need identification in OPPT's risk evaluation process for high-priority designated substances. The framework includes a decision tree where toxicity predictions by ECOSAR and data from analogues inform the criticality of each environmental hazard data need. This framework supports the alternative testing consideration under 4(h) for ecotoxicity data gathering, provides a means for using NAMs in support of or in place of issuing TSCA section 4 test orders. Examples from chemicals currently undergoing risk evaluation and a description of the process for submitting a NAM for inclusion on TSCA's approved list are also highlighted.

01.14.03 Adaptation of a Species Sensitivity Distribution Approach for the Use of New Approach Methodologies in Ecological Risk Assessment

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New approach methodologies (NAMs) have the potential to modernize ecological risk assessment but there are several barriers to their adoption by end-users. Key barriers to the uptake of NAMs in ecological risk assessment include determining which NAMs are sensitive and reliable indicators of hazard and how best to incorporate them into quantitative estimates of risk. For traditional endpoints, the potential for ecological risk is estimated by comparing a predicted no effect concentration (PNEC) to a predicted environmental concentration (PEC). For data-rich chemical substances, PNECs are preferentially derived from species sensitivity distributions (SSDs), with the PNEC equal to the concentration at which 5% of species are adversely affected. Some recent Canadian Water Quality Guidelines have been developed using a statistical approach that fits SSDs from several statistical distributions to derive a weighted model averaged PNEC. This approach is flexible and could be used to derive PNECs exclusively from NAM endpoints or from a combination of traditional and NAM endpoints. The approach would allow NAMs to be quantitatively incorporated into estimates of PNECs and the sensitivity of a NAM endpoint could be determined through a comparison with other endpoints in the same SSD. This presentation will discuss the conceptual framework for using a SSD approach that incorporates NAMs, its advantages and disadvantages, and the feasibility of the adoption of this approach in risk assessment.

01.14.04 Leveraging New Approach Methodologies to Complement Aquatic Life Criteria Derivation

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The USEPA's 1985 guidelines for the derivation of aquatic life criteria (ALC) are robust, but data intensive. This is one factor contributing to the fact that fewer than 100 ALC have been established, and values remain outstanding for many thousands of chemicals. Thus, alternative analyses/processes that can provide provisional values to guide states, tribes, and other stakeholders while data accumulate and more rigorous criteria are derived, would be beneficial. The overarching purpose of this study was to assess the feasibility of using data from new approach methodologies (NAMs) like ToxCast to derive first-pass, provisional values to guide chemical prioritization and resource management as a complement to traditional ALC derivation. To address this goal, the study objectives were to 1) estimate chemical potency using data from NAMs for four compounds with available ALC, 2) evaluate the utility of using NAMs data to elucidate potential mechanisms of toxicity to guide problem formulation, and 3) evaluate the species relevance/applicability of toxicity pathways for compounds with clearly defined mechanisms of action as a means to evaluate whether minimum data requirements could potentially be waived when deriving a more formal ALC. Briefly, points of departure were derived from ToxCast data based on the 5th percentile of the distribution of activity concentration above cutoff (ACC) values falling below the cytotoxic burst. Mechanistic inferences were made based on active target hits in ToxCast and, where applicable, assessed for taxonomic conservation using SeqAPASS. Without adjusting for uncertainty, ToxCast-based PODs for nonylphenol (14.5 µg/L), pentachlorophenol (193 µg/L), PFOA (347 µg/L), and PFOS (19.5 µg/L) aligned closely (within a factor of 10) with corresponding national water quality guidelines/criteria from the US (nonylphenol, pentachlorophenol), Canada (PFOS), or Australia (PFOA). Moreover, pathways of toxicity gleaned from the NAMs data were reflective of *in vivo*-based findings from the literature. These results, while preliminary, and based on a limited number of substances, support the potential application of NAMs data to complement traditional ALC derivation approaches and prioritization. *The contents of this abstract neither constitute, nor necessarily reflect, USEPA policy.*

01.14.05 Stakeholder Perspectives on the Uptake of New Approach Methodologies for the Assessment and Management of Chemical Substances

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Given current legislative mandates to assess the safety of thousands of chemicals and the slow pace at which conventional testing proceeds, a wide number of people, organizations and institutions envision a shift towards the use of New Approach Methodologies (NAMs) that promise to reduce costs and delays. However, despite various efforts by scientists, governments and the private sector to modernize toxicity testing, the formal adoption of NAMs in chemical risk assessment and management generally remains limited. This might be the case because insufficient attention has been paid to professional, organizational, institutional and political factors, despite previous studies pointing out the importance of these aspects. Using the theory of policy entrepreneurship, this study explores how change is realised in institutional environments that often resist change, what strategies and resources are used, which actors are critical in the process, and how, why and through what processes institutional and policy change can be generated. Methodologically, this study draws on data collected through 67 semi-structured interviews and 4 focus groups (with a total of 24 participants) conducted with experts in the ecotoxicology and chemical risk assessment and management community in Canada, the USA and Europe between July 2017 and May 2020. The findings reveal how policy and institutional change might be facilitated to accelerate advancements in the regulatory field. Recommendations for the uptake of NAMs in chemical risk assessment and management are discussed. This study is part of the EcoToxChip project (www.ecotoxchip.ca).

01.14.06 Eas-E Suite: An Integrated Platform for New Approach Methodologies to Facilitate Hazard, Exposure, and Risk Assessment

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New Approach Methods (NAMs) are being considered to address hazard and exposure data gaps contributing to the modernization of chemical screening, prioritization, and risk assessment. NAMs broadly include in vitro bioactivity data, in vitro-in vivo extrapolation (IVIVE), in silico (e.g., quantitative structure activity relationships (QSARs)), and informatic approaches (e.g., high-throughput toxicokinetics (HTTK) and exposure models) that can rapidly identify those chemicals that pose the greatest health concern. However, application of NAMs for scientific evaluations and decision-making requires knowledge of the applicability domain of available databases and models to provide reliable assessments. To facilitate the application of NAMs and bridge the gap between evolving scientific research and regulatory assessment challenges, we introduce the Exposure And Safety Estimation (EAS-E) Suite platform. EAS-E Suite is a free and publicly available system that seamlessly fuses various curated databases (e.g., physical-chemical properties, in vitro and in vivo TK data), QSARs, environmental fate, Physiologically Based Toxicokinetic (PBTK), and exposure and risk estimation models. EAS-E Suite includes curated, measured physical-chemical properties, environmental biodegradation, and biotransformation half-lives for > 50 thousand chemicals. EAS-E Suite also houses multi-media mass-balance environmental fate, bioaccumulation and exposure models, currently including the Risk Assessment IDentification And Ranking (RAIDAR), and RAIDAR-Indoor and Consumer Exposure (RAIDAR-ICE) models for simulating chemical fate in natural and indoor environments and aggregate exposures to representative ecological receptors and humans. EAS-E Suite also contains the Chemicals in Products - Comprehensive Anthropospheric Fate Estimation (CiP-CAFE) model for estimating chemical life cycle, mode-of-entry and emission rates. A general one-compartment physiologically based toxicokinetic (1Co-PBTK) model that can be parameterized to different mammals (e.g., human or rat) is implemented to allow HTTK simulations. With entry of only chemical structure, the system can select the best values to parameterize the different models and provide holistic ecological and human health assessments. Here we demonstrate how EAS-E Suite provides opportunities to address regulatory challenges for new and existing chemical assessments for ecological and human health objectives.

01.14.07 Addressing Applicability Domain and Uncertainty for In Vitro and In Silico Toxicokinetic for High Throughput Data Interpretation

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New Approach Methods (NAMs) such as in vitro bioactivity data, in vitro-in vivo extrapolation (IVIVE), high-throughput toxicokinetics (HTTK), and exposure models are contributing to the modernization of 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 for scientific evaluations and decision-making by establishing the applicability domains (AD) of existing HTTK models and databases. This presentation determines the AD and assesses the uncertainty of current IVIVE and HTTK data to estimate administered equivalent doses (AEDs) from in vitro bioactivity test data. Extensive in vivo, in vitro and in silico data sources for parameterizing, applying, and evaluating HTTK models were collected and compared. The in vitro and in vivo data were critically evaluated for data quality, and confidence scores are provided for each data point. All the required toxicokinetic parameters were obtained from empirical estimates, Quantitative Structure Activity Relationships (QSARs), and/or in vitro biotransformation rate assays and suitable IVIVE models. Three HTTK modelling approaches are considered and compared i) considering total elimination half life (HL_T) predicted by QSAR and a composition model for volume of distribution (VD_{SS}) to calculate steady state blood concentration (C_{SS}), ii) a general one-compartment physiologically based toxicokinetic (1Co-PBTK) model that can be parameterized to different mammals as implemented in the Exposure And Safety Estimation (EAS-E) Suite platform, iii) the "3 Compartments steady state" model developed by the US-EPA as implemented in the "httk" R package. The three approaches are applied to calculate HL_T and C_{SS} to identify their AD, particularly relative to their required input parameters to better understand the chemical space that can be covered by current HTTK models. The combination of EAS-E Suite tools and databases allows for HTTK simulations for 96% of the case study chemicals (n=12,123), whereas the EPA-HTTK could only be parameterized and applied to the 311 chemicals with empirical in vitro hepatic clearance rates. The results of this work generally support the computational IVIVE and HTTK methods required to estimate AEDs. General guidance and recommendations are provided for applying HTTK methods for AED calculations and improving HTTK modelling and risk estimation.

01.14.08 Determination of Chemical Partitioning in In Vivo Aquatic High-Throughput Assays

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The US Environmental Protection Agency (USEPA) is tasked with evaluating large numbers of chemicals for potential ecological effects. Traditional toxicity testing is time and resource intensive, and high throughput screening methods (e.g., USEPA's ToxCast program) have been developed to generate alternative data rapidly at lower cost. However, to date, these approaches have been almost entirely focused on human health and mammalian biology and may not adequately cover the diversity of pathways necessary to protect wildlife and plants. To fill this gap, ecologically relevant new approach methodologies (NAMs) that accommodate high throughput testing (HTT) of aquatic species in 96-well plate formats are under development and results are being compared with those from traditional toxicity assays. Understanding chemical behavior and partitioning in HTT format, particularly the free chemical concentration in solution, is critical for comparing standardized toxicity data with NAMs-derived data. In the present study, an existing model (Armitage model) was used to estimate disposition of 15 chemicals in *in vivo* aquatic HTT assays. The selected chemicals spanned a diverse range of physicochemical properties and known toxicological modes of action (e.g. phthalates, xenoestrogens, and organophosphates). Model parameters were compiled, and chemical disposition modeled using the Armitage model within the high throughput toxicokinetics (httk) R package. In addition to modeled values, a subset of compounds was empirically verified by liquid chromatography mass spectrometry (LC-MS) in pre- and post-exposure media to compare with model outputs. Optimized model input parameters, estimated chemical partitioning, and empirically measured concentrations will be presented and discussed and the overall effect on comparability of the results with traditional benchmarks reported. *The contents of this abstract neither constitute nor necessarily reflect USEPA policy.*

01.14.09 Fish Connectivity Mapping II: An Upgraded Library of Expression Profiles and Its Performance Evaluations

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Connectivity mapping (Cmap) links conditions, either of chemical or biological nature, through their respectively associated multi-gene queries and whole transcriptome rank-ordered gene lists (ROGLs) organized as a library. Cmap leverages an entire such library for chemical grouping, read-across, diagnostics, and discovery of mechanisms of action (MOAs). The limited Cmap library created in a 2016 pilot study has been significantly upgraded to encompass hundreds more microarray studies of fathead minnow and zebrafish available from the NCBI GEO repository as of March 2021. Also included is a small but increasing RNA-seq collection from ongoing toxicogenomics research at the EPA. The library now contains over 8000 ROGLs based on more than 11,000 samples, representing 600-plus unique chemical or biological conditions. To evaluate the toxicological applications of this library and its performance, we constructed more than 1100 query sets from the same studies to interrogate the library computationally. Performance evaluation was conducted base on a dozen chemical class or biological conditions found in the query sets with each containing multiple independent studies targeting a common condition. They ranged from dioxins, polycyclic aromatic hydrocarbons, estrogens, pesticides, to field samples. A pair of mapped query-ROGL was considered positive when both were associated with the same or similar chemical class/biological condition but derived from two independent studies. For each such condition, queries were expected to recover above a minimum (based on the number of selected studies of the same tissue/organ type) but below a maximum (all the selected studies assumed to have the same tissue/organ type) number of target ROGLs since tissue/organ type is critical to gene expression yet was often unknown. Overall, an average of 257% of minimum expected mappings was recovered successfully among the top five hits of individual ROGL collections. Also discovered were many other unexpected but positive ROGL targets, the matching queries of which were either not constructable from their original studies or not included in analysis. These findings have further demonstrated the utilities of Cmap in toxicology. This upgraded library marks another important step towards a better integration of toxicogenomics data in the future. As its coverage of chemicals and biology grows, so will the power of its various applications.

01.14.10 Investigating the Use of Artificial Intelligence to Predict the Wastewater Treatment System (WWTS) Removal Rates of Organic Chemicals

T.D. Burns, C. Inglis, Environment and Climate Change Canada / Ecological Assessment Division

The ecological risk assessment of a chemical relies upon exposure characterization, in which a predicted environmental concentration (PEC) is calculated and used to quantify the potential for organisms to be exposed to it. The wastewater treatment system (WWTS) removal rate is a key parameter used to calculate a PEC for the aquatic environment. In the absence of measured information, estimated WWTS removal rates can be derived from multimedia fugacity models such as EPI Suite (USEPA) or mechanistic models such as SimpleTreat (RIVM). New approach methodologies such as machine learning provide an alternative approach to estimating WWTS removal rates. To investigate this further, a gradient-boosted decision tree model was developed. The model was trained using measured WWTS removal rates for 125 organic chemicals and a suite of topological descriptors based on radial distribution functions to describe the various structural features of each chemical. This presentation will describe the preliminary outcomes from the resulting model, and the advantages and disadvantages of this approach.

01.14.11 Developing Hyalella azteca Embryo Toxicity Assay for High Throughput Toxicity Tests

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Toxicity tests are effective monitoring tools in evaluating site contamination and can be used to investigate how toxicants affect different organisms. Often times, toxicity tests are expensive, require a great number of samples, and use vertebrate model organisms. Due to these factors, there is a need to reduce reliance on vertebrate testing and develop new approach methodologies (NAM) that can achieve similar results in ecological risk assessments that are cost-effective and efficient. High throughput testing have been conducted for invertebrate species, however they have been highly underrepresented in NAMs. *Hyalella azteca* is a freshwater amphipod that is used in many ecotoxicology applications to evaluate the effect of pollutants in sediments and water ways. Many different toxicants have been evaluated for their effect on adult and juvenile *H. azteca*, however little has been studied using *H. azteca* embryos. The goals of the present study were to define endpoints of *H. azteca* embryonic development that could be routinely used to assess the toxicity of chemicals and to characterize the toxicity of several key pollutants on *H. azteca*. *H. azteca* embryonic development was characterized into six readily distinguishable stages for toxicant evaluation. *H. azteca* development could be monitored using embryos removed from the female brood chamber and incubated ex vivo in 6-well places at 23°C. Complete embryogenesis occurred for approximately 12 days and included the following stage milestones: Stage 1 – cell division stage, Stage 2 – soccer ball stage, Stage 3 – cell organization, Stage 4 – head and limb formation, Stage 5 – Digestive tube and eye completion, and Stage 6 – hatched *H. azteca*. Following evaluation of developmental endpoints, next steps include conducting 96 hour exposures of toxicants to *H. azteca* embryos to look at their impact on mortality, developmental rate, or any phenotypic changes upon hatching. Suggested phenotypic changes include deformities to the head lobe and digestive tube regions, as well as halted eye development. The high throughput embryonic toxicity test hopes to evaluate many pollutants and environmentally relevant mixtures of pollutants on survival and development of *H. azteca* that can be further applied to environmental assessments and regulations.

01.14.12 Comprehensive Assessments of Oolichan (Thaleichthys pacificus) Population Health and Range Using Molecular Tools in Impacted Aquatic Spawning Habitat

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Protection of threatened aquatic species relies on timely, reliable, and detailed monitoring data, which can be highly challenging for species whose life histories are not well described. Oolichan (Eulachon: *Thaleichthys pacificus*), an anadromous smelt with a discontinuous spawning range from California to Alaska, has experienced steeply declining population numbers over the past few decades and the cause of this has not yet been clearly defined. In this study we assess Oolichan populations on the Central Coast in Haisla territory near Kitimat, BC, which have seen significant recent development including a new oil processing plant and increased marine traffic, using a newly developed and highly sensitive environmental DNA (eDNA) assay for the purpose of detection and abundance correlation of Oolichan. In addition, we perform transcriptomic analysis of larvae and non-lethal fin clips using RNA-seq and quantitative real time polymerase chain reaction methods to address the need for more effective population health assessment methods. Caudal fin clips have successfully been used to identify and characterize exposure to water soluble components of marine oil spills. By comparing specimens from spawning habitats with different levels of ecological impact, combined with rigorous abundance estimates using eDNA sampling methods, we aim to describe species population numbers, range, and health to a degree of detail that is impossible without this new generation of cutting edge, non-invasive molecular tools.

01.15 Understanding Diversity in Species Sensitivity to Chemicals

01.15.02 Toxicokinetic and Phylogenetic Profiles for Explanation of Species Differences in Sensitivity to Acetaminophen Among Bird Species

P. van den Hurk, Clemson University / Biological Sciences

Previous research has revealed that snake species are extremely sensitive to acetaminophen (APAP). A relatively small amount of this common non-steroidal anti-inflammatory drug will kill snake species within 24 h. We discovered that the underlying mechanism is that snake species are lacking the specific isoform of the glucuronosyltransferases (UGT) that conjugates and detoxifies the acetaminophen. In addition, snakes are missing the N-acetyltransferase enzyme that detoxifies the APAP metabolite aminophenol, which is binds to hemoglobin causing met-hemoglobinemia and kills the snake through hypoxia. For this reason, APAP is used to control snakes when they become a nuisance as invasive species, like the brown treesnake on the island of Guam. However, while APAP is relatively non-toxic to most mammalian species, the question has come up if acetaminophen is also toxic to bird species, which may be problematic when APAP-laced bait is distributed in the environment to control the invasive snake population. In a novel approach to predict the toxicity of APAP in bird species, chickens were exposed to increasing acetaminophen doses, ranging from 80 to 325 mg per day for four days. No mortality or abnormal behavior was observed during the exposure, and after 4 days liver samples were collected to measure upregulation of bird-specific UGTs, together with blood, liver, bile and kidney samples. Liver samples were processed to extract mRNA, which was analyzed with qPCR primers previously developed for UGT isoforms in bird species. Upregulation of specific isoforms did reveal which bird UGT isoforms are involved in APAP biotransformation. The analysis of plasma and bile samples by UPLC/MS showed which APAP metabolites are formed in chickens and how these metabolites are excreted. This information supports the qPCR data about which UGT isoforms are responsible for the APAP metabolism in chickens. Comparison of the expression profile will then be used to evaluate if other bird species are able to express the critical UGT isoforms, which will enable the prediction if other bird species can metabolize and detoxify acetaminophen. This information will be used by environmental managers to establish safe snake management strategies and protect reintroduced bird species in locations where invasive snakes have eliminated those bird species.

01.15.03 Comparison of Hepatic Transcriptomic Responses to Ethinylestradiol in Embryonic Japanese Quail and Double-Crested Cormorant

Y. Jeon, McGill University / Natural Resource Sciences; D. Crump, Environment and Climate Change Canada / National Wildlife Research Centre; J. Ewald, E. Boulanger, McGill University / Natural Resource Sciences; P. Liu, McGill University / Institute of Parasitology; J. Xia, McGill University Macdonald Campus; N. Basu, McGill University / Faculty of Agricultural and Environmental Sciences; M. Hecker, University of Saskatchewan / School of the Environment & Sustainability and Toxicology Centre; J. Head, McGill University / Natural Resource Sciences

Species difference in susceptibility to toxicants is a critical issue in ecotoxicology. Transcriptomics is being proposed as a strategy to improve toxicity testing for regulatory risk assessment; however, different responses among species have not been extensively explored using the transcriptomic approach. In the present study, we identify dysregulated genes and pathways in Japanese quail (JQ) and double-crested cormorant (DCCO) embryos following exposure to ethinylestradiol (EE2). The objectives were to evaluate unique and overlapping transcriptomic responses to estrogenic exposure in the two species, and to determine whether these responses correspond to previously reported sensitivity difference at the organismal level. 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, 3.33, and 33.3 µg/g egg weight. After 9-days (JQ) or 16-days (DCCO) of incubation (representing mid-incubation stage), liver tissues were collected from 5 embryos per treatment group for RNA-sequencing. Data were processed using EcoOmicsAnalyst (dev.ecoomicsanalyst.ca), in which a KEGG Orthology database for all birds and an assembled transcriptome of each species together served as a reference database. Then, using NetworkAnalyst (www.networkanalyst.ca), differential expression analysis and gene set enrichment analysis were performed using DESeq2 and EcoToxModules, respectively. Following exposure to EE2, JQ and DCCO had 8 and 562 KEGG orthologs differentially expressed, respectively, having three of them in common. An EcoToxModule of PPAR signalling was enriched for both species. The results of this study will contribute to a better understanding of the estrogenic effects of contaminants in birds, the expansion of taxonomic coverage in chemical risk assessment from model to non-model species, and the evaluation of transcriptomics and early life stage tests as alternative toxicity testing methods. This study was conducted as a part of a large-scale Genome Canada-funded project, EcoToxChip project (www.ecotoxchip.ca).

01.15.04 Interspecies Variation in Sensitivity to Activation of the Aryl Hydrocarbon Receptor by Polycyclic Aromatic Hydrocarbons in Zebrafish, Japanese Medaka, and Fathead Minnow

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Polycyclic aromatic hydrocarbons (PAHs) are a diverse group of chemicals characterized by the presence of 2 or more fused aromatic rings. Although PAHs are naturally occurring, extraction processes and human activity increase loading of PAHs into aquatic systems. Fishes can suffer a variety of toxicities due to activation of the aryl hydrocarbon receptor (AhR) by PAHs. To date, most research on fishes has focused on zebrafish *(Danio rerio),* but the sensitivity of zebrafish may not be representative of other fishes due to interspecies variation in sensitivity to AhR activation by PAHs. To address this question, a luciferase reporter gene assay was performed using AhR expression constructs from 3 phylogenetically diverse model species: zebrafish, Japanese medaka *(Oryzias latipes)*, and fathead minnow *(Pimephales promelas).* The assay tested AhR activation of each species by benz(α)anthracene (BAA) or 1 of 3 alkyl derivatives: 4-methylbenz(α)anthracene (4MBAA), 8-methylbenz(α)anthracene (8MBAA), and 7,12-dimethylbenz(α)anthracene (7,12DMBAA). The half maximal effect concentration (EC_{50}) for BAA was highest in zebrafish at 2.23 nM, and lowest for fathead minnow at 0.188 nM. The EC_{50} of Japanese medaka fell in the middle at 0.638 nM. The EC_{50}s for 4MBAA followed the same pattern, with zebrafish being the least sensitive at 0.842 nM, followed by medaka at 0.249 nM, and fathead minnow being the most sensitive at 0.236 nM. The most potent of the tested chemicals, 8MBAA, had an EC_{50} of 0.198 nM in zebrafish, 0.121 nM in medaka, and 0.165 nM in fathead minnow. Lastly, the least potent of the tested chemicals, 7,12DMBAA, had an EC_{50} of 6.210 nM in zebrafish, 2.504 nM in medaka, and 3.777 nM in fathead minnow. Zebrafish were the least sensitive of the investigated species to activation of the AhR by every tested chemical, but it is not yet known whether this will translate to *in vivo* sensitivity. To validate the *in vitro* AhR activation data, ongoing research will expose embryos of each species to each PAH using micro-injection. The median lethal dose (LD_{50}) of each PAH at the transition to exogenous feeding will be compared across species and used to expand understanding of interspecies variation in sensitivity to activation of the AhR by PAHs by determining the relationship between sensitivity to *in* *vivo* toxicity and sensitivity to *in vitro* AhR activation. This relationship will be used for informing the selection of native species of concern for subsequent studies.

01.15.05 Can Blood Protein Diversity Among Fish Species Help Explain Perfluoroalkyl Acid Trophodynamics in Aquatic Food Webs?

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Perfluoroalkyl acid (PFAA) trophodynamic studies on aquatic food webs have generally produced inconsistent and inconclusive evidence of trophic magnification. Both controlled uptake experiments and peculiarities involving enhanced accumulation of PFAAs in lower trophic level organisms suggest that aspects beyond direct trophic transfer govern PFAA trophodynamics in these systems. For biota, PFAA accumulation, distribution, and elimination depend on several protein-ligand interactions. In blood, PFAAs exist almost exclusively in protein complexes, typically due to their relatively high affinity for serum albumin, an abundant serum protein in many organisms which transports fatty acids. Previous publications suggest relatively high diversity among fish species' blood proteins, including the absence of albumin in some species. Studies of both mammal and fish blood proteins indicate that lipoproteins serve as fatty acid transporters in the absence of albumin. Toxicokinetic modeling efforts suggest that PFAA serum transport protein-related parameters are the most important in determining PFAA fate within fish, yet fish proteomes are largely uncharacterized. Ongoing work seeks to identify if serum protein variability among three Lake Ontario fish species (alewife (*Alosa pseudoharengus*), deepwater sculpin (*Myoxocephalus thompsonii*), and lake trout (*Salvelinus namaycush*)) may contribute to PFAA trophodynamics in this aquatic food web. Preliminary gel electrophoresis and proteomics experiments revealed general differences among these species' serum proteomes. Work in progress aims to 1) identify interspecies differences in PFAA-binding serum proteins and 2) discern to what degree binding capacity and/or strength of PFAA affinity towards these serum proteins varies among these three species.

01.15.07 Comparing the Functional Equivalency of Natural and Created Wetlands to Promote Regional Amphibian Biodiversity and to Inform Proactive Land Management

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Wetlands provide ecological functionality by maintaining and promoting regional biodiversity supporting quality habitat for aquatic organisms. Globally, habitat loss, fragmentation and degradation due to increases in agricultural activities and urban development have reduced or altered geographically isolated wetlands, reducing biodiversity. The overall objective of this study was to compare the functional equivalency of natural wetlands (natural ponds) and created wetlands (excavated ponds and stormwater basins) in the New Jersey Pinelands by comparing wetland hydrology, water quality (including pesticide concentrations), and plant and anuran assemblages. Twenty-four wetlands were chosen and further classified by land use into reference (minimum land-use effect) and degraded (maximum land-use effect). This presentation will highlight relationships found by statistically comparing (a) tadpole and (b) adult anuran species distribution across wetlands with habitat variables that displayed covariation between wetland types and strong correlations with biogeographic species groups. Abiotic and biotic wetland variables were similar between natural and excavated ponds despite wetland classification (reference vs degraded), with notable differences between the ponds and stormwater basins. Natural and excavated ponds displayed characteristic Pinelands water quality (low pH range from 4.0 to 6.1, high organic carbon), exhibited high ecological integrity and thus supported native anuran and plant assemblages. Whereas stormwater basins exhibited degraded water quality (pH range from 5.8 to 7.5, elevated pesticide concentrations) and were dominated by non-native and introduced plant and anuran species. As development increases and natural wetland connectivity is lost, proactive local land management could include creating wetlands that offer the characteristic habitat of naturally occurring wetlands on the landscape. Increasing quality habitat is vital for conservation of native amphibians within their current biogeographic ranges.

01.16 Environmental Toxicology and Stress Response

01.16.01 A Multi-Compartment Toxicokinetic Model for the Evaluation of Per- and Polyfluoroalkyl Substance Concentrations Within Developing Zebrafish (Danio rerio)

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Per- and polyfluoroalkyl substances (PFAS) are contaminants of concern for installations across the US Department of Defense. The long-term stability of PFAS and their bioaccumulation nature creates a very complex bioremediation problem for organisms affected by environmental contamination. Even at lower levels of PFAS exposure, many aquatic species exhibit adverse physiological outcomes across the development cycle. Data gaps relating PFAS exposure to its tissue distribution in developing organisms complicate our capability to proactively assess the long-term risks of exposure in the environment. To address this problem, we developed a toxicokinetic model of the developing zebrafish (*Danio rerio*) to permit transformation of aqueous PFAS exposures into tissue-specific PFAS concentrations. Our model describes PFAS absorption, distribution, and membrane adsorption throughout the developing zebrafish from 0 to 120 hours post fertilization (hpf), and describes accumulation within the chorion, yolk, and embryo, both before and after hatching. During early development, two of the most relevant physiological phenomena to affect PFAS toxicokinetics include: (i) the dynamic volume of the zebrafish, and (ii) the presence of the chorion and its absence upon hatching around 48 hpf. Current toxicokinetic modeling approaches do not adequately account for both. To address (i), we assume that energy budgets are biased toward growth, from which we developed an ordinary differential equation model to describe the volume of the perivitelline space, embryo, and yolk compartments as a function of time. To address effects associated with how the chorion is shed during the hatching event (ii), we employ a "population averaging" approach to account for a mixture of both hatched and unhatched zebrafish through time. Our toxicokinetic model parameters are fit from literature data, and the model incorporates a number of proposed mechanistic interactions, including PFAS with lipid membranes, to describe the rate-limiting steps of accumulation at the cellular level of tissues. As such, the model can be mechanistically linked with data from transwell assays to predict intracellular PFAS accumulation and maintain physiological and mass balance rigor, which provides a solid foundation for quantitative adverse outcome modeling at the level of the molecular initiating event.

01.16.02 Assessing the Role of Soil Nitrogen Content on the Interactions Between Perfluorinated Carboxylic Acids (PFCAs), Lettuce (Lactia salvia), and Radishes (Raphanus sativus)

N. Legge, University of Guelph

Per- and polyfluoroalkyl substances (PFAS) are a group of anthropogenic chemicals that have been used in industry for the past 60 years. The strong carbon-fluorine bonds make these pollutants environmentally persistent, chemically and thermally stable, and extremely resistant to degradation under ambient conditions. PFAS are ubiquitous across all environmental compartments and geographies and can bioaccumulate leading to concerns regarding long-term human and environmental health. Chronic exposure to PFAS causes genotoxicity, neurotoxicology, endocrine disruption, weakened immunity, and may increase the severity of the Covid-19 infection. Human consumption of crops grown in contaminated soil is one of the main entry points for PFAS into the human food chain. Therefore, understanding the relationship between PFAS, crops, and soil, is essential in identifying the risk that PFAS have on humans. One factor that may influence plant uptake of PFAS is soil nitrogen content. Studies on artichoke and cabbage found that increasing soil nitrogen can enhance the plant protein content by 26%, which may have implications for increased plant uptake of PFAS, as PFAS strongly interacts with protein. With the high use of nitrogen in agriculture, in combination with the ubiquity of PFAS and its high affinity for protein, the consumption of crops grown in contaminated soil may pose increased risks to human health. Therefore, this study aims to understand plant response to PFAS in agroecosystems and how soil nitrogen content can influence this relationship. These objectives will be addressed by applying environmentally relevant concentrations of PFAS and varying concentrations of soil nitrogen to lettuce and radishes. Samples will be analyzed using Nuclear Magnetic Resonance spectroscopy (NMR) based metabolomics. An increase in the plant stress response is expected when plants are exposed to high soil nitrogen and PFAS. The plant stress response will be identified based on significant perturbations to plant metabolic cycles and associated alterations to the concentration of plant metabolites when comparing treatment and controls spectra. This research is essential in understanding the relationship between PFAS and agroecosystems and can aid in forming solutions to decrease human exposure to PFAS through crop consumption. Future research could be conducted on the impacts that different organic matter constituents have on the relationship between PFAS and crops.

01.16.03 Development of a Robust In Silico Bioconcentration Factor (BCF) Model Based on Ionization State Distribution to Promote Sustainability in Chemical Manufacturing

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Experimental measurement of Bioconcentration Factor (BCF), a key measure 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 $D_{pH=7.4}$). We hypothesized that this shortcoming could be due to the fact that existing models do not explicitly capture the distribution of charged species for chemicals at physiological pH, which is known to significantly affect bioavailability. Herein we show that by modeling physicochemical properties of all relevant charged species at biological pH we can develop a model that significantly improves prediction of BCF for many chemical classes, including the pharmaceutical chemical space. Overall, we demonstrate that the explicit consideration of ionization could be applicable to improving other predictive models that improve our ability to assess ecotoxicity and other endpoints of commercial chemicals.

01.16.06 Effects of Embryonic Exposure to Aroclor 1254 on Neurologic, Cardiac, Growth, and Reproductive Endpoints in Zebrafish

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Polychlorinated biphenyls (PCBs) are long-lived synthetic compounds that were widely used until 1979. We investigated the effects of exposure to Aroclor 1254 in zebrafish embryos and correlated them to tissue concentrations. Embryos were exposed at 6 hpf via aqueous solution for 96 hr without renewal. Nominal concentrations of Aroclor 1254 ranged from 6% to 700% of measured concentrations. Tissue (embryo) samples were collected at 36 hpf for congener analysis. We collected samples at 102 hpf for RNA-Seq analysis. Heart rate and a neurological endpoint (eye tremors) were measured at both 102 and 174 hpf, and cardiac edema was assessed at 102 hpf. Growth was assessed every two weeks after 1 mpf and reproductive trials started at 4 mpf. Cardiac edema was not present; however, dose-dependent bradycardia was observed at 102 hpf. Similarly, a dose dependent increase in eye tremor duration was observed at both 102 and 174 hpf. Eye tremor behavior appears similar to that of other dopaminergic-related neurodegeneration associated with PCB exposure. Bioinformatic analyses found that the top biological processes affected included visual function pathways supporting the eye tremor phenotypes. These findings are important in relating a novel neurotoxic endpoint to tissue concentrations of PCB in early life stage fishes.

01.16.07 Effects of Maternal Bisphenol a Exposure on Hypothalamic Vasopressinergic Circuit in Mice

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Bisphenol A (BPA), a well-recognized endocrine disruptor that has been linked to numerous adverse outcomes, is ubiquitously detected in humans, including pregnant women. Increasing epidemiological and animal studies showed associations between prenatal BPA exposure and social-behavioral issues in childhood. Since vasopressinergic circuit plays important role in regulating social behaviors, and our previous studies showed that prenatal exposure to BPA altered vasopressin development in offspring. Herein, we proposed to evaluate the effects of maternal BPA exposure (2.25 µg/kg body weight, even lower than that recommended by the European Food and Safety Agency) on vasopressinergic circuit including the number of arginine vasopressin (AVP) neurons in hypothalamic subregions, AVP projections to intra- and extra-hypothalamic regions across the brain and the electrophysiological properties of AVP neurons using immunohistochemistry, whole-brain imaging and whole-cell patch clamp. Our results showed that maternal BPA exposure increased the number of AVP neurons in paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus. Besides, we found in female mice that maternal BPA exposure increased the intra-hypothalamic projections, including the PVN-SON projection and the projection to the retrochiasmatic nucleus, while opposite effects were found in males. Disrupted projections to extra-hypothalamic regions, including lateral septum, bed nucleus of the stria terminalis, amygdala and lateral habenula, were also observed in a sex specific manner by BPA exposures. In addition, our whole-cell patch clamp data showed that female AVP neurons maternally exposed to BPA have lower spontaneous action potentials (sAP) frequency than that of the control neurons, while male AVP neurons exhibited higher sAP frequency. We also observed that AVP neurons from

BPA group were less responsive to current injections than the neurons from control group, indicated by fewer neuronal spikes, delayed latency to the first spike and smaller proportion of responsive neurons. Spontaneous excitatory post-synaptic currents frequency and amplitude were also altered by maternal BPA exposure in females but not in males, while no significant changes were found for spontaneous inhibitory post-synaptic currents in neither females nor males. Collectively, our results suggest that low dose maternal BPA exposure might disturb vasopressinergic circuit across the brain with a sex difference.

01.16.10 Examining Metabolic Consequences of Toxicological Exposures in Resilient Urban Corals Under Global Change Stressors

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Marine organisms located downstream from urban centers face potentially negative interactive effects from pollutants (heavy metals, microplastics, pharmaceuticals, increased nutrients) and global change stressors (increasing sea temperature and acidity). Corals are just one example of a marine urban organism that may be vulnerable to these stressors. One seemingly resilient urban coral is Astrangia poculata, which exists along the eastern United States coast from Massachusetts to Florida at a range of temperatures and conditions, including the New York Harbor. Though it appears to be surviving in these habitats in the field, it is unclear if the coral will be resilient to the impacts of urban pollutants and global sea surface temperature increases, or how different life history stages will respond. We present preliminary findings on the effects of microplastics (pollutant vector) and caffeine (pollutant indicator) under normal and elevated temperatures on the metabolic activity of the urban coral A. poculata in three life stages: adults, larvae, and eggs. Understanding the effect of these stressors on early life stages (egg and larvae) will allow researchers to evaluate whether urban pollutants or increased temperatures will reduce coral fecundity and fitness, which may ultimately lead to population reductions. During summer 2021, A. poculata individuals were exposed to field-measured and elevated levels of caffeine (ng/uL), microplastics, and combinations thereof simultaneously with ambient (22°C) and elevated (30°C) temperatures. Metabolic activity (measured via oxygen consumption on a respirometer) was used as a read-out to determine sublethal response to stressors. Overall, we aim to establish dose-response relationships for each stressor (caffeine, microplastics, and temperature) and combination thereof at each life stage, providing insight into the sensitivity threshold of this resilient urban marine organism. Our study tests the hypothesis that A. poculata will undergo metabolic changes under exposure to caffeine-laden microplastics, with higher mortality and metabolic depression at higher temperatures and earlier life history stages. A. poculata co-exists with commercially important species such as oysters, clams, scallops, crabs, and mussels, thus results from this study have significant environmental toxicology and human health implications.

01.16.11 Exposure of Caenorhabditis elegans (C. elegans) to Electronic Cigarette (E-cig) Constituents Produces Degeneration of Dopaminergic Neurons

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Electronic cigarettes (e-cigs) are battery-powered devices that generate aerosols from a heated liquid and are a popular alternative to tobacco products, particularly among youths. While nicotine, a component of tobacco found in traditional cigarettes and many types of e-cigs, is recognized as a toxic and addictive chemical known to alter brain function, there exists very little information regarding the specific neurotoxic effects of other e-cig constituents either alone or in combination. Thus, an *ex vivo* study was carried out using green fluorescent protein-enabled (GFP) dopaminergic pathway-labeled *Caenorhabditis elegans* (*C. elegans*), to fill this gap. Using this well-established alternative model to study neuronal morphology and degeneration, first larval (L1) stage *C. elegans* worms were treated in nematode growth media (NGM) for 48hr with the humectants, propylene glycol (PG) and vegetable glycerin (VG), alone and in combination (i.e., PG/VG) and with and without different dilutions of nicotine, to assess any neurodegenerative effects. Exposure to high concentrations of either PG, VG, PG plus VG (5% and10%) or nicotine alone (30mM) altered worm behavior by reducing movement speed in a dose-dependent manner. Changes in neuronal morphology were also observed 48 hr. following treatment with media containing either PG alone, VG alone, PG plus VG, nicotine alone or nicotine combined with PG/VG. Treatment with either PG or nicotine alone caused the most severe effects on neurodegeneration based on our lab-developed neuronal scoring criteria. Accordingly, each worm was scored for the absence (0) or presence of morphological changes representing degeneration including: thinning of neuron projections (1); 2-3 bleb formations (2); >3 bleb formations or shrunken soma (3); and loss or breaks in GFP (4). Overall, our findings indicate that direct treatment of *C. elegans* with either humectant alone and with and without nicotine causes the degeneration of dopaminergic neurons similar to that reported for traditional cigarettes. These data clearly demonstrate that e-cig constituents, other than nicotine, can be just as dangerous for brain health as nicotine itself, particularly for vulnerable adolescent populations. This translational study provides additional evidence for the neurotoxicity of e-cig components that could be used for setting policy to protect public health. Supported by the NYU P30 CIEH Center (5P30ES000260-51) and NIEHS R01ES10563.

01.16.12 Genetic Polymorphisms in Resident and Migratory Turkey Vultures Upon Lead Induced Oxidative Stress

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Turkey vultures (*Cathartas aura*), a key species in many ecological communities' food webs, often feed on carcasses left behind by hunters who use lead (Pb) shot, resulting in their exposure to this toxic metal. In humans, genetic polymorphisms are associated with variation in susceptibility to the toxic effects of Pb. No information exists about the genetic variation in susceptibility to Pb in vultures or any other birds. Due to turkey vultures' long-standing exposure to Pb, a population genetic response may also be expected, due to selection upon these genetic polymorphisms. Recent data on Pb blood concentrations and antioxidant activities in vultures from CA will allow me to test for how differences in the susceptibility to Pb are associated with SNPs in four specific genes. Further, the use of museum DNA samples will allow me to investigate whether an evolutionary response to Pb exposure has occurred in recent decades. Aminolevulinate Dehydratase (*ALAD*), *RAC2*, Glutathione Peroxidase (*GPx1*), and Metallothionein 1A (*MT1A*) genes are some of the identified potential candidate genes, due to their role in lead transport and oxidative stress. ALAD, a gene that encodes for the ALAD enzyme and aids in heme production, is inhibited by Pb whereas the GPx1, RAC2, and MT1A genes play a key role in reducing reactive oxygen species, protecting the cell against oxidative damage, and detoxification of heavy metals in the cell, respectively. Therefore, testing for SNPs in these four candidate genes will allow me to possibly unveil genetic variation in susceptibility to Pb in turkey vultures.

01.16.13 Identification of Toxic Contaminants Affecting the Endangered St. Lawrence Estuary Beluga Population

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The beluga (*Delphinapterus leucas*) population of the St. Lawrence Estuary (SLE) is threatened by non-recovery, and chronic exposure to contaminants, including persistent organic pollutants (POPs), is considered a major underlying cause. While elevated tissue concentrations of POPs have been reported in SLE belugas, traditional targeted chemical analyses and toxicity testing strategies are limited in their capacity to identify the causative chemicals; in particular, they may overlook unknown compounds. There is therefore a pressing need to develop unbiased and robust methodologies to effectively identify the contaminants that may be posing significant risks to this population. In light of this, our study aimed to apply nontargeted analysis (NTA) to identify contaminants from SLE beluga tissue. NTA of beluga liver extracts (collected from animals found stranded between 2000-2017) under electrospray ionization revealed the presence of 54 known and unknown per- and polyfluoroalkyl substances (PFAS) at high concentrations (Σ_{54}PFAS = 113.8 – 5391 ng/g ww), and highly abundant perfluoroalkyl sulfonamides were of particular concern due to their known toxicity. Atmospheric pressure chemical ionization was employed to detect further POPs in liver and blubber, which revealed ~100 known and unknown halogenated contaminants, including organochlorine pesticides. Encouraged by this, we applied pooled liver and blubber extracts (100 g_{tissue}/mL) in an *in vitro* aryl hydrocarbon receptor (AhR) bioassay (H4IIE-Luc rat hepatoma cell line) 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 beluga blubber extracts exerted significantly higher AhR activity than a reference population from the Canadian arctic, indicating that a unique chemical profile in SLE beluga tissue may be responsible for underlying nuclear receptor-mediated adverse health pathways. Ongoing efforts are focused on characterizing the contribution of known and unknown chemicals towards AhR activity. Ultimately, the results of this study will help fill critical knowledge gaps regarding the health risks posed to the SLE beluga population and will support future conservation strategies.

01.16.14 In Vitro Test to Detect the Presence of Neurotoxic Compounds in Water and Sediment Samples

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In this work, an *In Vitro* test for the detection of compounds with neurotoxic effects in water and sediment samples is proposed, since *In Vitro* tests have been considered as an alternative to animal tests, in order to comply with what was proposed in the proposal of the 3 Rs (Replace, Refine and Reduce). In addition these techniques have the advantages of having a better control in the conditions in an experiment, reduce the ethical implications, and are of lower cost. In this proposal, a method was developed using mollusc foot tissue, exposed to water and sediment samples to evaluate the activity of the AchE enzyme, to detect the presence of compounds with neurotoxic effects. Tests were carried out with metals (Cd, Cu, Cr and Pb), surfactants (LAS) and pesticides (Dichlorvos and Cypermethrin), to evaluate the response of this *In Vitro* test to these contaminants. The tissue homogenates were prepared with phosphate buffer (pH 7.2), then the homogenate was incubated with the contaminants for 30 min. And then the activity of the AchE enzyme was determined by the Ellman method (microplate). The results obtained with the contaminants were significantly different from the tests without contaminants. In the tests with cadmium (0.1, 0.5 and 1.0 mg / L) inhibits of the activity of AchE were registered from 8.5%, to 22.4%, in the tests with detergents (0.05, 0.1, 0.5 mg/l) from 11.5 to 32.7% and in the tests with pesticides from 23.8 to 86.5%, in all cases a response according to the concentration of the contaminants was observed.

01.16.15 Modeling the Long-Term Effects of Chemicals in the Honey Bee Colony: A Comparison of a Mechanistic Modeling (GUTS) and a Probit-Based Approach

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Honey bees are regularly exposed to trace levels of various chemicals in the environment, which may persist in their colonies over prolonged periods of time. In contrast, our understanding of chemical toxicity in honey bees is primarily based on brief (2-4 day) assays, which are required during pesticide registration. Recently, longer assays (up to 10 days) are also required, but there's no standard approach in place for extrapolating the results into longer timescales of exposure (weeks or months). To address this, there's high demand for modeling approaches that can predict the cumulative effects of chemicals to honey bee colonies over time. In the last decade, the General Unified Thresholds Model of Survival (GUTS) has emerged as a state-of-the-art model of toxicity, capable of extrapolating survival data from laboratory trials into time-variable and long-term exposure scenarios. For the present study, I incorporated GUTS into a simple honey bee population model. Using the model, I simulate the exposure of colonies to chemicals that are either known or suspected of causing time-cumulative toxicity (certain metals and systemic pesticides). I compare a GUTS-based modeling approach with a more "traditional" probit-based approach, finding that these approaches often reach overlapping predictions. Cases where modeling approaches disagree indicate areas for future work. This work demonstrates how different approaches to modeling toxic effects can be incorporated into honey bee population models for chemical risk assessment as well as basic research in the area of honey bee ecotoxicology.

01.16.16 Phenotypically Anchored mRNA and miRNA Expression Profiling in Zebrafish Reveals Flame Retardant Chemical Toxicity Networks

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The ubiquitous use of flame retardant chemicals (FRCs) in the manufacture of many consumer products leads to inevitable environmental releases and human exposures. Studying toxic effects of FRCs as a group is challenging since they widely differ in physicochemical properties. We previously used zebrafish as a model to screen 61 representative FRCs and showed that many induced behavioral and teratogenic effects, with aryl phosphates identified as the most active. In this study, we selected 10 FRCs belonging to diverse physicochemical classes and zebrafish toxicity profiles to identify the gene expression responses following exposures. For each FRC, we executed paired mRNA-micro-RNA (miR) sequencing, which enabled us to study mRNA expression patterns and investigate the role of miRs as posttranscriptional regulators of gene expression. We found widespread disruption of mRNA and miR expression across several FRCs. Neurodevelopment was a key disrupted biological process across multiple FRCs and was corroborated by behavioral deficits. Several mRNAs (e.g., osbpl2a) and miRs (e.g., mir-125b-5p), showed differential expression common to multiple FRCs (10 and 7 respectively). These common miRs were also predicted to regulate a network of differentially expressed genes with diverse functions, including apoptosis, neurodevelopment, lipid regulation and inflammation. Commonly disrupted transcription factors (TFs) such as retinoic acid receptor, retinoid X receptor, and vitamin D regulator were predicted to regulate a wide network of differentially expressed mRNAs across a majority of the FRCs. Many of the differential mRNA-TF and mRNA-miR pairs were predicted to play important roles in development as well as cancer signaling. Specific comparisons between TBBPA and its derivative TBBPA-DBPE showed contrasting gene expression patterns that corroborated with their phenotypic profiles. The newer generation FRCs such as IPP and TCEP produced distinct gene expression changes compared to the legacy FRC BDE-47. Our study is the first to establish a mRNA-miR-TF regulatory network across a large group of structurally diverse FRCs and diverse phenotypic responses. The purpose was to discover common and unique biological targets that will help us understand mechanisms of action for these important chemicals and establish this approach as an important tool for better understanding toxic effects of environmental contaminants.

01.16.17 PhyloTox: An Approach to Identify Molecular Responses to Chemical Stresses

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PhyloTox is a phylogenetic toxicological approach that uses a diverse suite of animal models to identify the functions of evolutionarily conserved groups of genes, metabolites, and biomolecular interactions. We are developing a proof-of-concept for chemical safety assessment that will help to solve the enormous public health crisis caused by human exposure to chemical pollutants. PhyloTox draws insights on gene functions by disrupting entire networks through chemical exposure with distantly related organisms. Through the highly interdisciplinary mix of genomics, metabolomics, evolutionary theory, bioinformatics and toxicology, we can better classify the potential effects across the Tree of Life based on the evolutionarily conserved molecular pathways of response. In our current work, toxicological assays of chemicals, e.g., arsenic, on model organisms were conducted to establish concentration thresholds, where toxic benchmarks were determined. Metabolomic responses that were chemically induced were quantitatively measured for several organisms, e.g., zebrafish, fruit flies, and water fleas, with nano-electrospray-direct infusion-mass spectrometry (nESI-DI-MS). Significantly changing metabolites were revealed through machine-learning computational approaches and further characterized by targeted tandem MS analysis. With the cluster and structural information, chemically affected pathways were statistically assessed. To conclude, we have established a workflow for metabolomics related to the PhyloTox approach, and future work will correlate the observed metabolic changes with transcriptomic responses.

01.16.18 Physiological and Metabolomic Changes in Plants Exposed to Perfluorooctanesulfonic Acid (PFOS)

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Perfluorooctanesulfonic acid (PFOS) is a widely used, environmentally persistent, perfluorinated sulfonic acid. PFOS has a wide range of uses from being a fire retardant to application resulting in non-stick surfaces. The presence of PFOS in the environment has raised questions about health concerns and how it effects terrestrial plants, especially food crops. This study looks at the physiological responses, elemental uptake, and metabolite profile of velvetleaf (*Abutilon theophrasti*) and wheat (*Triticum aestivum L.*) after long-term exposure to different PFOS concentrations (0, 25, and 50 mg/kg soil). Results showed very similar responses for both velvetleaf and wheat in several parameters that were measured such as plant height, root, shoot and seed dry masses, chlorophyll content, stress regulations, macro and micronutrient contents, and metabolite profile.

01.16.19 Possible Bias of the Circadian Rhythm on the Transcriptional Response of Juvenile Yellow Perch (Perca flavescens) Exposed to Clothianidin and Chlorantraniliprole

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Neonicotinoid pesticides, including clothianidin, are widely used in agriculture to fight pest insects. Given the upcoming restrictions on neonicotinoid use in Canada, new replacement compounds such as the diamide chlorantraniliprole are increasingly used. Concentrations reaching up to 100-200 ng/L of clothianidin and chlorantraniliprole have been measured in surface water samples from Lake St. Pierre, Quebec, Canada. However, toxicity data on non-target aquatic organisms such as fish remain scarce for these compounds. Juvenile yellow perch (*Perca flavescens*) inhabit macrophyte beds and floodplains of Lake St. Pierre and are therefore prone to pesticide exposure in agricultural areas. The goal of this study was to evaluate the effects and modes of action of clothianidin and chlorantraniliprole in juvenile yellow perch chronically exposed *in vivo* (28 days) to environmental concentrations (200 ng/L) of the two pesticides alone and in a mixture. Hepatic transcriptional responses were measured by RNA-sequencing and verified by qRT-PCR. Results showed that clothianidin did not affect gene transcription, while chlorantraniliprole deregulated the transcription of 37 genes, most of which were up-regulated. When the two compounds were combined, 251 genes were deregulated but no synergistic or additive effect were observed. Enrichment gene sets with the largest number of genes were related to circadian rhythmic processes for chlorantraniliprole alone and in a mixture. Normal daily rhythmic variation of clock-regulated gene transcription might have influenced the observed transcriptional response in perch due to differences in sampling time. Experimental validation was performed in juvenile rainbow trout (*Oncorhynchus mykiss*) using qRT-PCR to measure the transcription of circadian gene orthologs; results showed similar daily variation in mRNA levels of three genes (*cipc*, *clock*, and *per1*), suggesting that sampling time could have influenced transcriptional profiles. Altogether, results indicate that clothianidin and chlorantraniliprole had most likely no transcriptional effects in juvenile yellow perch exposed *in vivo* to environmental concentrations.

01.16.20 qPCR Analysis of Hemoglobin Modulation in Response of Co-Exposure to Hypoxia and Lead on Daphnia magna

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Developing an understanding of how stressors interact is critical in assessing environmental toxicity. Hypoxia is a stressor that impacts both fresh and saltwater ecosystems that is increasing in severity and prevalence due to anthropogenic factors. Lead (Pb) is a non-essential metal and pervasive pollutant that represents an additional source of stress in aquatic environments. Little is known regarding the potential interactive effects of co-exposure to hypoxia and Pb in aquatic animals. Our study focuses on the acute effects of co-exposure to hypoxia and Pb on *Daphnia magna*. In response to hypoxia, *D. magna* has been shown to elevate ventilation and heart rates. Furthermore, they can not only drastically increase hemoglobin (Hb) concentration in low oxygen conditions but also oxygen affinity by upregulating certain isoforms of hemoglobin of which they have seven. In contrast, exposure to Pb has been shown to decrease Hb synthesis. The significance of Hb in convective oxygen transport within *D. magna* presumably changes with development, becoming more important with growth beyond the critical size limit for purely diffusive oxygen transport (< 1 mm in normoxia). Thus, sensitivity to Pb might be related to oxygen transport effects that likely vary with age-related size differences and oxygen availability. To investigate the role of isoform switching, quantitative PCR (qPCR) was utilized to identify which isoforms of hemoglobin were expressed for different life stages of *D. magna* (neonates and adults) after acute (48-h) exposure using Pb exposures in normoxia and hypoxia. To gain insight into the potential role of Pb-induced impairment on convective oxygen transport in contributing to the observed toxicity, Hb concentrations and oxygen consumption rates were measured. This data should aid in predicting the toxicity of Pb to *D. magna* in conditions of environmental hypoxia.

01.16.21 Quantitation of Lipid Accumulation and Analysis of Lipid-Related Gene Expression Following Aqueous Film-Forming Foam (AFFF) Exposure in Human and Rat Liver Cells

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Aqueous film-forming foams (AFFF) are chemical mixtures known to quickly suppress hydrocarbon fires, but their use and emissions have resulted in the contamination of bodies of water used for drinking water consumption with persistent chemicals called per- and polyfluoroalkyl substances (PFAS). PFAS found in contaminated water have been associated with adverse liver outcomes, particularly non-alcoholic fatty liver diseases (NAFLD), and disrupted blood lipids. We hypothesized that PFAS in AFFF are causing hepatic lipid accumulation and initiating pathways related to liver disease. Differentiated 2D cultures of HepaRG human liver cells were exposed to 5 contemporary AFFF using blinded allocation for 7 days at a concentration range of 0.0002% to 0.2% (v/v). Additionally, HepaRG cells were also exposed to 9 AFFF constituents, which included both fluorinated and non-fluorinated compounds, known to be present within these AFFF formulations. Lipid accumulation was measured using an Oil Red O stain specific to neutral triglycerides. Lipid accumulation was quantitated from cell morphology photomicrographs using the open-source image analysis software Fiji, which measured the average fluorescence intensity of all the cells per image. Assays were conducted in triplicate, and three biological replicates. At the lowest exposure concentration of 0.0002%, each AFFF produced a 4-6 fold increase in fluorescence intensity relative to vehicle control cells. In a separate study, Sprague Dawley rats (n=10/dose) were exposed to these 5 AFFF daily in both a 1-day and 14-day study. PCR analysis was performed with rat liver cDNA using an initial set of 8 genes (*Acox1, Cyp4a1, Cyp2b1, Cyp2b2, Cyp3a1, Cyp3a2, Adipoq and Plin2*) involved in fatty acid oxidation and lipogenesis, with particular focus on the PPAR, CAR, and PXR pathways. One of the 5 AFFFs produced notable responses, approximately two-fold expression increases or decreases occurring for each of the 8 genes, building over 24 hours with PPAR and diminishing with PXR after 14 days, which is consistent with findings in PFAS literature. Exposure of 3 out of the 5 AFFF was observed to have an effect in up-regulating the CYP gene superfamily specifically during the 1-day exposure at 0.03%, 0.3%, and 1% concentrations, but these responses diminished after 14 days of exposure at these same concentrations. These data suggest that PFAS and nonfluorinated components do play a role in promoting lipid accumulation in human liver.

01.16.22 Role of Prdx1 in Mediating Diquat and Tert-Butyl Hydroperoxide-Induced Oxidative Stress in Zebrafish Larvae

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The peroxiredoxins (Prdxs) are a ubiquitous family of cytosolic antioxidant proteins that are conserved among vertebrates. As a major member of 2-Cys Prdx subfamily, the Prdx1 protein, uses thioredoxin as an electron donor and protects cells from oxidative damage to cellular DNA, lipids and proteins by scavenging H_2O_2 and other reactive oxygen species. Furthermore, we have shown that the *prdx1* mRNA is highly inducible in zebrafish larvae and under control of the transcription factor Nrf2a, suggesting a protective role against oxidative stress. However, while Prdx1 has been well studied in human and animal cells, little is known about the role of this protein in protecting against pollutant toxicity in fish. To this end, we used CRISPR/Cas9 technology to generate three *prdx1* knockout zebrafish lines and examined the protective role of Prdx1 against two model oxidative stressors, including the redox cycling herbicide diquat, and the direct acting oxidant *tert*-butyl hydroperoxide (tBHP). Zebrafish larvae lacking Prdx1 were observed to be both viable and fertile. However, after 72 hr exposure to diquat (5 mg/L) or tBHP (90 mg/L), *prdx1*$^{-/-}$larvae displayed significantly higher mortality rates than observed for wild type (WT) zebrafish. The mRNA levels of *prdx1* increased in zebrafish larvae during exposures to both compounds, indicating an upregulation by Nrf2a in response to diquat and tBHP-induced cellular oxidative stress. The effect of *prdx1* genotype status on expression of other sublethal endpoints of oxidative stress in the presence of chemical exposures is ongoing and will further inform the mechanism of Prdx1 protection in fish. Collectively, our studies indicate that Prdx1 is an important and understudied component of the zebrafish antioxidant defense system that protects against chemical-induced oxidative stress. Supported by NIH Superfund ES004696.

01.16.26 The Role of Paraoxonase 1 in Metabolic Disruption: A Zebrafish Feeding Trial

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Endocrine disrupting chemicals have long been studied for their effects on steroid hormone pathways. Recent studies have shown that some endocrine disruptors also have a metabolic disrupting effect, which could result in fat accumulation in liver tissue when lipid metabolism is disturbed. However, the details of these interactions are still not fully understood. One such interaction is between these compounds and paraoxonase 1 (PON1), an enzyme with antioxidant functions, known to be associated with fatty liver and to metabolize xenobiotic substances, particularly organophosphate pesticides such as chlorpyrifos. In order to better understand the mechanism by which PON1 affects aberrant fat accumulation under the influence of metabolic disrupting chemicals, zebrafish are exposed via food to vehicle, bisphenol A and chlorpyrifos. Half of each exposure group receives a normal diet, while the other half is fed a high-fat diet for 6 weeks to simulate circumstances in which fatty liver would develop. During the exposure, weight and length of the fish is followed up. After 6 weeks, PON1 activities, lipid peroxidation, toxicant concentration, energy reserves and mRNA expression of genes involved in energy metabolism or downstream effects of bisphenol A and chlorpyrifos are measured in liver tissue to characterize the relations between these parameters. This characterization gives insight into the mechanisms of metabolic disruption and the role of oxidative stress and biotransformation of pollutants.

01.16.27 Toxicity of Firefighting Water Additives to Terrestrial and Aquatic Biota

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The use of firefighting water additives has greatly increased over past decades in both wildland and residential fires in order to enhance traditional water application firefighting methods and increase extinguishing efficacy. Fluorinated fire-fighting additives were used extensively in the past, however, due to their bioaccumulative potential and persistence in the environment they are no longer permitted in Canada for forest and residential applications. With greater concern of the environmental fate of firefighting water additives, new formulations have been developed that are meant to be “eco- friendly” alternatives for fire suppression. There currently exists very little data on the toxicity of additives in current use with respect to terrestrial and aquatic biota. This study will assess the toxic effects of nine different types of firefighting water additives on terrestrial and aquatic species. This will include acute lethality testing of the aquatic species *Daphnia magna*. The terrestrial portion will include chronic testing for the springtail species *Folsomia candida* and germination and emergence tests for three plant species (*Picea glauca, Agropyron cristatum*, and *Raphanus sativus*). The *D. magna* portion of the study revealed considerable risk for all tested products with the exception of Eco-gel and TetraKO. The *F. candida* tests revealed relative sensitivity to all products with the exception of TetraKO and Bio FOR N. *Picea glauca* and *A. cristatum* germination tests showed relatively high sensitivity at the lowest administered concentrations for all tested products, whereas *R. sativus* showed relatively low sensitivity to all tested products with the exception of LC95A. Finally, emergence tests with *R. sativus* also showed relatively high sensitivity to LC95A. The results of this study highlight the potential hazard that firefighting water additives pose to aquatic and terrestrial organisms.

01.16.28 Toxicological Assessment of Novel Nanoparticle Used for Oil Spill Remediation Using Fathead Minnow Embryos and Juveniles As a Model

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Oil exposure to fish due to spill accidents is a concern and different remediation strategies have been proposed. We proposed the use of Nanotechnology to engineer a novel nanosized particle (NP) that can function as a unimolecular micelle, which can facilitate the oil slick breakdown, plus entrapping the oil for degradation. In this project, we evaluated the toxicity of this NP by exposing fathead minnow embryos and juveniles to different concentrations of NPs. The NP consisted of a silica core, grafted with amphiphilic chemical branches, all with known biocompatibility. The hypothesis was that the addition of NP will help prevent the exposure of fish to oil components, hence, lowering expression of biomarkers of oil exposure. The endpoints evaluated were mortality, gene expression of cyp1a, and the EROD assay. Furthermore, an RNAseq analysis was conducted to explore the different pathways activated or mitigated before and after the addition of NP to the water-accommodated fraction (WAF) of the oil. The mortality test showed low toxicity for both life stages after 96 h of exposure. The expression of cyp1a gene showed a decrease after the addition of NPs to the WAF solution, suggesting that the NPs were reducing the bioavailability of the oil components. Furthermore, the EROD assay also showed a decrease, which confirmed that CYP1A protein concentration was not increased in fish that were exposed to a combination of NPs and WAF, compared to controls exposed only to WAF. Among enriched pathways, gene expression related to necrosis was increased in embryos exposed to WAF alone, as expected. This enriched pathway was not present in embryos treated with WAF+NPs, suggesting that the NPs diminished the bioavailability of components found in WAF. Only one pathway, the glutamate release pathway, was enriched in both the WAF alone and the WAF+NPs. Our results suggest that this novel NP is a good candidate to be used in future oil spill remediation.

01.16.29 Transcriptomic Investigation of a 4 Fluorinated Carbon Homologous Series of Per- and Polyfluoroalkyl Substances (PFASs) in Zebrafish

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Per- and polyfluoroalkyl substances (PFASs) have been used in a variety of consumer and industrial products. Many are ubiquitously detected in the environment and humans, and some induce toxic effects. While studies have proposed various modes of action through which PFAS may induce toxicity, additional investigation is needed. Additionally, most studies have focused on just a handful of PFASs. Given that thousands of structurally unique PFAS exist, there is a need for toxicological investigation across structural subclasses. In this study, we leveraged developmental zebrafish as a complex *in vivo* model to elucidate mode of action through transcriptomics in a homologous series of PFAS with 4 fluorinated carbons and different functional head groups (sulfonamides, sulfonic acids, carboxylic acids, and fluorotelomer sulfonates). Perfluorobutane sulfonamide (FBSA), perfluorobutane sulfonic acid (PFBS), perfluoropentanoic acid (PFPeA), and 4:2 fluorotelomer sulfonic acid (4:2 FTS) were evaluated for developmental toxicity across a range of 0-100 μM. Zebrafish were statically exposed in 96-well plates beginning at 6-8 hours post fertilization (hpf), and mortality and morphological endpoints were assessed at 24 and 120 hpf. Only FBSA induced significant incidence of combined any effect, enabling concentration-response modeling and calculation of an effective concentration for 80% incidence (EC_{80}). Therefore, the EC_{80} of FBSA (47 μM) was selected as the exposure concentration for all PFAS during the transcriptomic investigation. Zebrafish were exposed as previously described but collected at 48 hpf, before observable morphological effects, for RNA extraction. RNA-sequencing was conducted using the DNBseq platform. Exposure to FBSA elicited 1,909 differentially expressed genes (DEGs). The most highly enriched biological pathways based on gene ontology analysis were related to lipid metabolism and cellular homeostasis. PFBS, PFPeA, and 4:2 FTS exposure elicited far fewer DEGs, ranging from 3 to 14. Considering both morphology and transcriptomics, we identified the sulfonamide PFAS as most bioactive in the homologous series. Our findings provide insight into FBSA mode of toxic action, support the concept that transcriptomic effects are predictive of morphological effects, and will be compared to transcriptomic investigations of additional bioactive PFAS in the future.

01.16.30 Uncertainties in Estimating Effect Concentrations in Aquatic Toxicity Tests

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For chronic toxicity to aquatic organisms, the past 30 years have seen a growing interest in the estimation of concentrations causing a certain percentage effect (ECp) rather than using highest no-observed effect concentrations (HNOEC) or lowest observed effect concentrations (LOEC) as the basis for risk characterizations. ECp estimation has certain clear advantages for better defining risk. However, especially for low values for "p", an ECp can be highly uncertain due to various issues regarding estimation methodology, model selection, and data sufficiency. To define and illustrate these issues, we conducted a "megatest" of the chronic toxicity of NaCl to *Ceriodaphnia dubia*, involving a much higher-than-normal number of treatment concentrations and replicates. This dataset is used to illustrate uncertainties that can occur for ECp estimation of more typical tests, especially for estimation of low effect levels. *This abstract does not necessarily reflect U.S. EPA policy.*

01.16.31 Urban Soil Bacterial Shifts and Its Influence on 2,4-D Fate and Breakdown

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The herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) is a common pesticide used on urban landscapes in the U.S. Although 2,4-D fate and breakdown have been well documented, interactions with the entire soil microbiome and simultaneous assessments of its transformation products (TPs) in urban soils remain poorly understood. Soil bacteria are the primary drivers of pesticide transformation. Seasonal environmental variations are key factors that directly influence soil bacterial community structure and function, which may alter pesticide degradation networks resulting in the formation of TPs. The 2,4-D-degrading gene, *tfdA* (Class

I, II, and III), has been identified in soil bacteria and serves as a good marker for 2,4-D biodegradation activity. Our study evaluated the impact of seasonal environmental variation on the pesticide-degrading activity of soil bacteria and the formation of 2,4-D's main TP, 2,4-dichlorophenol (2,4-DCP), in two field sites in Madison, WI during Spring and Summer seasons. Alterations of the general soil bacterial community structure and pesticide-degrading activity were assessed using high-throughput sequencing analysis and *tfdA* gene class quantification. Our results suggest that bacterial community composition was distinct between field sites, soil layers, and months, with *Proteobacteria* and *Actinobacteria* being highly abundant. Xenobiotic degradation pathways were also predicted and correlated with 2,4-D degradation. Further, *tfdA* Class I and II were highly upregulated in 2018 after 2,4-D application. Similarly, *tfdA* Class II was also upregulated in 2019. Liquid Chromatography tandem Mass Spectrometry was used to detect and quantify 2,4-D and its TPs. Preliminary results show detection of 2,4-D of 5-20 ug g^{-1} in leaf less than 1 ppm in leachate samples. In soil samples, 2,4-D peak detection was on Day 0 post-application. 2,4-DCP was not detected in leaf, leachate, or soil samples. These findings will ensure better intensive plant and pest management strategies landscapes of high quality with fewer non-target impacts on environmental and human health and enhance our overall understanding of 2,4-D metabolism and its main TPs.

01.16.32 Using Zebrafish Embryo As a Model to Study the Biological Effects of 5G-Level Radiofrequency Radiations

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The rapid deployment of 5G spectrum by the telecommunication industry is intended to promotebetter connectivity and data integration among various industries. However, since exposures toradio frequency radiations (RFR) >2.4 GHz are increasing, concerns about their potentialhealth impacts are ongoing. In this study, we used the embryonic zebrafish model to assess theimpacts of a 3.5 GHz RFR on biology- a frequency that is used by 5G-enabled cell phones andlies within the 4G and 5G bandwidth. We established a plate-based exposure setup for RFRs,exposed developing zebrafish to 3.5 GHz RFR, specific absorption rate (SAR) ≈ 8.27 W/Kg from6 h post fertilization (hpf) to 48 hpf, and measured a battery of morphological and behavioralendpoints, transcriptomic responses and adult behavioral effects. Our results revealed- 1) nosignificant impacts on mortality, morphology or photomotor response and a modest inhibition ofstartle response suggesting some levels of sensorimotor disruptions, 2) perturbation of geneexpression during development suggesting metabolic disruptions and 3) subtle disruption of freeswimming and shoaling behavior in adults. These indicate that the cell phone radiations at lowGHz-level frequencies could induce subtle, yet persistent sensorimotor effects. Importantly,we have now established a robust setup for zebrafish RFR exposures readily amenable to testingvarious powers and frequencies. Future studies will aim at investigating RFR impacts on themetabolome as well as study effects at higher frequencies.

01.20 Late Breaking Science: Environmental Toxicology and Stress Response

01.20.01 Abstract Title: Pre-Exposure Fecundity in the Fish Short Term Reproduction Assay With Fathead Minnow

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To investigate endocrine disrupting properties of plant protection product active ingredients in non-target organisms, the fish short term reproduction assay (FSTRA, OECD TG 229; OCSPP 890.1350) is a recommended *in vivo* study to identify activity on the estrogen, androgen, and steroidogenesis (EAS) modalities. The fathead minnow (*Pimephales promelas*) is the only species acceptable under both guidelines and there are several guideline differences. In addition to EAS mechanistic endpoints, the FSTRA also evaluates fecundity, which should be sufficiently high in all replicates at initiation to provide adequate statistical power as a test endpoint. To initiate a OECD 229 FSTRA, it is suggested that during the 7-14 day pre-exposure phase >10 eggs/female/day are obtained in each replicate tank. In contrast, the OCSPP 890.1350 guideline requires a minimum of 15 eggs/female/day per replicate as a validity criterion for fathead minnow. Mean historical control fecundity data for fathead minnow is ~23 eggs/female/day (Wheeler et al. 2019), based primarily on studies conducted in US labs. To examine pre-exposure fecundity in fathead minnow, data were collected from four initiated FSTRA studies conducted at European labs over the last 2 years. The results show that after a pre-exposure phase of 7 days (or longer), the minimum recommended fecundity of >10 eggs/female/day in OECD 229 was not achieved in sufficient replicate tanks to initiate the test, although all fish were apparently mature and healthy. Egg production was much lower compared to fathead minnow fecundity data based on OSCPP requirements and historical control data. The variability in fathead minnow fecundity might be attributed to differences in laboratory fish sources, housing conditions, and/or varying personnel experience. Moving forward, it will continue to be important to understand and document the practical experience gained using OECD 229 with fathead minnow globally to help inform further guidance on endocrine disruption testing for non-target organisms.

01.20.02 Accumulation of Micro and Nanoplastic Particles in Biomphalaria Glabrata (Mollusca, Gastropoda, Planorbidae) and the Subsequent Induction of Host Defense Responses

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Gastropods, widely distributed in the aquatic environment, are being used as a tool of toxicity assessment for a wide range of chemicals, nanomaterials, and other pollutants. Bioaccumulation of environmental pollutants is common in Gastropods because of the filtration systems employed by the Gastropods for food ingestion. However, studies on the toxic effects of the contaminants on the embryonic stages of Gastropods are limited. The present study utilizes the embryo of a common Gastropoda species, *Biomphalaria glabrata*, to reveal the accumulation and toxic effects of a widely distributed aquatic pollutant, micro/nanoplastic particles (MNPs), on the embryonic development and host defense of *B. glabrata*. The goal of this study is to identify the relationship between the sizes of MNPs and their bioaccumulation in *B. glabrata* embryos, as well as the host responses of the embryos to MNP stimulation. The embryos of *B. glabrata* were produced in the laboratory conditions and treated with various sizes of polystyrene MNPs (0.03µm, 0.5µm, and 1.0µm) that were fluorescently labeled through modification of amine groups on the surface of MNPs. The acute toxicity of PNPs to the development of embryos was evaluated by the mortality rate and hatching rate of embryos. The absorption of MNPs by *B. glabrata* embryos was estimated by measuring fluorescent intensities that remained in the water after MNP treatment. The entry and retention of MNPs in *B. glabrata* embryos were visualized using fluorescent microscopic imaging. Genetic responses of embryos to MNPs treatment were evaluated using quantitative polymerase chain reaction (qPCR) on some common stress-response genes. The result of the study showed that the presence of MNPs during the embryonic development of *B. glabrata* increased the mortality rates and hatching rates (or time). Fluorescent imaging has shown the MNPs do accumulate on the surface of the egg mass and accumulation did increase over the duration of the treatment. Results for gene transcript quantification demonstrated up-regulation in several stress response genes, such as heat shock protein 70, glutathione S-transferase, and cytochrome P450 in embryos when they were treated by 0.03µm MNPs for 96 and 120 hours. This study suggested that the smaller MNPs could have more severe impacts on the development of Gastropod embryos. This animal model can be further developed for the assessment of MNP toxicity in the aquatic environment.

01.20.03 Active Biomonitoring for Per- and Polyfluoroalkyl Substances in the Occoquan River Watershed, Virginia Using the Invasive Asian Clam Corbicula fluminea

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Considered contaminants of emerging concern, per- and polyfluoroalkyl substances (PFAS) are a class of toxic, manufactured chemicals found in commercial and consumer products such as nonstick cookware, food packaging, and firefighting foams. Their chemical composition makes them resistant to environmental degradation, mobile in groundwater, and bioaccumulate in living organisms. Recent monitoring efforts found high concentrations of PFAS in Northern Virginia drinking water sourced from the Occoquan River. PFAS could originate within the Occoquan watershed from industrial facilities, military bases, municipal wastewater treatment plants, and non-point sources such as septic systems and stormwater. Another potential explanation for PFAS levels in this watershed is an aqueous film-forming foam (AFFF) spill at Manassas regional airport in February 2020. This current study tests the reliability of using the invasive Asian clam *Corbicula* as a bioindicator for PFAS in surface water and sediment within the Occoquan River watershed. *Corbicula* are invasive, abundant bivalve mollusks and sedentary filter feeders that live at the water-sediment interface in aquatic environments. Known for high filtration rates and tolerance of toxicants, *Corbicula* is recognized as a suitable bioindicator for water and sediment quality. Sampling locations were grouped into three areas: (1) upstream from a regional water reclamation plant, (2) between the reclamation plant and a drinking water treatment plant, and (3) downstream from the drinking water treatment plant. The design of this study is intended to validate PFAS extraction using micro-QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) and document levels of PFAS in the watershed. Initial sampling occurred in September 2021. Extractions using micro-QuEChERS and analysis of PFAS using liquid chromatography-tandem mass spectrometry (LC-MS/MS) are ongoing. We hypothesize that PFAS is primarily conserved in *Corbicula* tissue and thus represents bioaccumulation over a two-year period, which, combined with sediment and water measurements, will aid in identifying recent and ongoing sources of PFAS in the Occoquan watershed. We hypothesize that we will find the lowest PFAS concentrations at the most upstream sites and the highest at the furthest downstream sites. Thus, it may be essential to identify sources of PFAS in the Occoquan watershed to implement preventative measures.

01.20.04 Assessment of Cardiovascular Toxicity of tris(2-chloroethyl) Phosphate (TCEP) on Ex-Ovo Chicken Embryos by In Situ Observation and Transcriptome Analysis

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Tris(2-chloroethyl) phosphate (TCEP) is one of organophosphate flame retardants (OPFRs) that have been used in textiles, industrial materials and furniture to delay the spread of fire after ignition. TCEP has been detected in the tissues and eggs of birds. However, there are few studies regarding the effects of TCEP on avian embryos. The aims of this study were to assess the cardiovascular toxicity of TCEP exposure on *ex-ovo* chicken embryos by *in situ* observation and cardiac transcriptome analysis and to predict its adverse outcome pathways (AOPs). Chicken (*Gallus gallus domesticus*) embryos were exposed with graded doses of TCEP (50, 250, and 500 nmol/g egg) or DMSO on incubation day 0. After 56–60 hrs of incubation, each embryo was transferred to a shell-less incubation vessel. Toxic endpoints in chicken embryos were consecutively monitored *in situ* on 3rd to 5th day of incubation, and the heart rate and the length of extraembryonic blood vessels were measured by imaging analysis. On the 5th incubation day, total RNA was extracted from the heart and analyzed with a next-generation sequencing. Based on the differentially expressed genes (DEGs), we performed the enrichment analyses of the transcription factor, KEGG pathway, and Gene Ontology (GO). The heart rate was reduced in a dose-dependent manner on 5th incubation day. The length of extraembryonic blood vessels was significantly decreased on 4th incubation day in 250 and 500 nmol/g egg TCEP-treated groups. The transcription factor enrichment analysis intimated that the suppression of PAX4, PDX1, CTNNB1, FOXO3, STAT3, and TCF7L2 by TCEP exposure were critical molecular initiating events. The KEGG pathway enrichment analysis suggested that the insulin and VEGF signaling pathways were affected by TCEP exposure. AKT1, PIK3CA, and PIK3R3 were involved in both the insulin and VEGF signaling pathways, and their expression levels were significantly decreased in a TCEP dose-dependent manner. Moreover, six enriched GO terms associated with the cardiac conduction were found in TCEP-treated groups. The expression levels of eight DEGs related to the cardiac conduction, including BIN1 and CACNA1G, were significantly decreased in a dose-dependent manner. These results indicate that TCEP exposure to chicken embryos may alter the expression of genes related to the cardiac conduction (BIN1 and CACNA1G) and VEGF signaling (AKT1, PIK3CA, and PIK3R3), and consequently reduce the heart rate and blood vessel growth.

01.20.05 Biotransformation Modulates the Penetration of Metallic Nanomaterials Across the Blood Brain Barrier

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Although brain is protected by a tight physiological guardian named blood brain barrier (BBB), deposition of engineered nanomaterials (ENMs) in brain and consequent neurotoxicity has been reported. To date, it is still unclear whether and how ENMs enter the brain by crossing the BBB. Understanding the potential of ENMs to cross the BBB as a function of their physicochemical properties and subsequent behavior, fate, and adverse effect beyond that point is vital for evaluating the neurological effects arising from their unintentional entry into the brain, which is yet to be fully explored. This is not only due to the complex nature of the brain but also the existing analytical limitations for characterization and quantification of NMs in the complex brain environment. Herein, we conducted an interdisciplinary study by using a novel analytical workflow and *in vitro* BBB model, as a complex biological barrier, to determine and quantify the biotransformation of metallic NMs as a function of their physicochemical properties and correlate the influence of the biotransformation to the BBB-penetration ability and transport pathways. We found metallic ENMs transform in the BBB as affected by their shape, size and intrinsic solubility, which in turn modulates their transport form, efficiency and pathways through the BBB, and consequently their neurotoxicity. Very little was transcytosed to the basolateral (brain) side of the BBB model, with significant amounts being recycled back to the apical (bloodstream) side and limited retention in the BBB cells. Paracellular transport was only observed at the higher concentration tested and was associated with membrane damage and NM dissolution (Figure 1). The generated data about biotransformation modulated uptake and transport of NMs through BBB open a new horizon for medical application of NMs, e.g. targetable drug delivery systems for brain diseases and also for biological fate assessment of NMs in brain to support their risk assessment.

01.20.06 Comparative Analysis of Optical Toxicity of Four Per- and Polyfluoroalkyl Substances in Zebrafish Larvae

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Per- and polyfluoroalkyl substances (PFAS) have been widely used in various industries since the 1950s, but they are potentially capable of inducing a broad range of adverse health effects. Owing to strong regulations on legacy PFAS (i.e. PFOS and PFOA), the increasing use of short-chained alternative PFAS (i.e. PFBS and PFBA) has been also detected in environmental matrices. Recently, an epidemiologic study revealed the association between PFAS exposure and some eye diseases in human. However, limited experimental studies on potential of PFASs in optical toxicity have conducted. In this study, we performed the phototactic behavior assays to assess optical toxicity in zebrafish larvae after exposure to four PFAS. Zebrafish embryos were exposed from 2-4 hpf (hours post fertilization) to 120 hpf at 1, 10, 100 and 1000 µg/L of them. Additional exposure concentrations of 0.1 µg/L for PFOS and of 10, 50 and 100 mg/L for PFOA, PFBS, PFBA were considered to evaluate their potency. There were significant changes in response to the light stimulation compared with the control group by 0.34, 0.66 and 0.62-fold in PFOS-exposed groups at 10, 100 and 1000 µg/L, respectively. For PFOA exposure, the reduction was detected by 0.29-fold at 100 mg/L. These results indicated that PFAS cause the impairment of visual function in zebrafish larvae by inhibiting visually-mediated behavioral responses, and PFOS is highly potent for optical toxicity, followed by PFOA.

01.20.07 Light Dullness and Memory Deficit Caused by Butylparaben in Adult Zebrafish

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Neurotoxic effects of butylparaben (BuP), a well-known endocrine-disrupting chemical, have been studied poorly. In this study, a total of 144 male zebrafish was exposed to 0 (0.1 % DMSO), 0.01, 0.1, and 1.0 mg/L BuP for 28 days and neurobehavior tests (i.e., novel tank test, photomotor response test, and T-maze test) and molecular analysis (i.e., RNA-seq analysis, neurotransmitter and neurosteroid assays) were assessed with brain samples. The photosensitivity was drastically injured with a significantly reversed pattern in the 1.0 mg/L BuP-exposed group. The deficits of memory were shown at 0.1 mg/L and 1.0 mg/L groups. In RNA-seq data analysis using GO terms and KEGG pathway, neuroactive ligand-receptor interaction in GABAA receptor, phototransduction, and tight junction in the brain were significantly altered at 1.0 mg/L BuP-exposed group. Phototransduction signal occurs in the pineal gland and acts a key role in regulating circadian rhythm responding to light cues. Impairments in the pineal gland may be the main reason for the loss of photosensitivity. Allopregnanolone, which is a GABAA receptor agonist, in the neurosteroid system was significantly reduced. Consequentially, we suggest that BuP perturb GABAA receptor reactivity, light perception signaling, and penetrate the blood-brain barrier by disturbing the tight junction, resulting in abnormal responses toward light stimulation and memory impairment.

01.20.08 Masculinization of Female Mosquitofish From Communities Using Reuse Is More Extreme Than in Communities Using Septic Tanks

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Industrialization has led to an increase in hormonally active synthetic chemicals being introduced into the environment. These chemicals, known as Endocrine Disrupting Chemicals, or EDCs, disrupt the endocrine system in humans, as well as in many aquatic wildlife. Sources of the EDC's include pesticides, pharmaceuticals, personal care products and plasticizers. When exposed to androgenic chemicals, or EDCs, the anal fin of female mosquitofish (*Gambusia holbrooki)* can become elongated and develop into a gonopodium-like structure, which is naturally only seen in male. Since the 1980's masculinized female mosquitofish has been documented worldwide in different waterways, primarily downstream from wastewater treatment facilities and pulp mill effluents. In southwest Florida there are many older communities still using septic tanks for their wastewater treatment: in addition, many newer communities are piping in reuse water from wastewater treatment facilities for irrigation of yards, golf courses and other turf including verges on roadways and in parks. For this study, we compared the anal fin morphology of the female mosquitofish, *Gambusia holbrooki,* collected from surface waters in a community using septic for their wastewater to anal fins from fish collected from a community using reuse water for irrigation. We found that female fish showed higher degrees of masculinization in stormwater lakes of the community using reuse water systems than the drainage canals in the community using septic tanks. While we are still developing the methodology to be able to identify and quantify the precise chemicals present in the waters, we are assuming endocrine disruptors are the cause for this masculinization. We believe this is the first study of this type being done in Florida or comparing septic and reclaimed/ reuse water systems. Our findings emphasize the importance of understanding the implications of EDCs being present in our environment. We believe there is adequate evidence to urge better treatment of wastewater at facilities to prevent the release of potential EDCs at their source in our local environment for the safety of both humans and wildlife.

01.20.09 Microbiota Dysbiosis of Adult Zebrafish Exposed to Chlorinated Persistent Organic Pollutants (C-POPs) Mixture

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Chlorinated persistent organic pollutants (C-POPs) such as organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) are known to be associated with obesity, metabolic dysfunction risk, and gut microbiota dysbiosis. However, studies on alteration in the gut microbiota under exposed to a mixture of C-POPs are limited. In this study, we exposed adult zebrafish to 0.02, 0.1 and 0.5 µg/L of C-POPs mixture (i.e. 5 OCPs and Aroclor 1254 with 1:1 ratio) for 12 weeks. Gut microbiota were analyzed by selecting five dissected intestine samples in the order of glucose levels measured at each concentration. The V3-V4 region of the 16S rRNA genes were sequenced by the Illumina Miseq Sequencing system. There were no significant differences in body weight and body length between control and exposed groups. In the hematological study, glucose levels were significantly increased at 0.02 and 0.1 µg/L. As a result of gut microbiota analysis, Fusobacteria (59.65-78.20%) was the highest proportion in all groups, followed by Proteobacteria and Bacteroidetes. The relative abundance of Fusobacteria decreased at 0.02 and 0.1 µg/L compared to the control group. At the genus level, Cetobacterium was the most abundant genus in all groups. Alpha diversity including Chao 1 and ACE indexes were significantly reduced in zebrafish exposed to the C-POPs mixture compared to the control at 0.02 and 0.5 µg/L. These results suggest that dysbiosis of gut microbiota was companied with elevated glucose level under exposure to C-POPs mixture in zebrafish.

01.20.10 OECD Test No. 249: Fish Cell Line Acute Toxicity - the RTgill-W1 Cell Line Assay: Development and Application

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The RTgill-W1 cell line assay, which is based on an immortal cell line of rainbow trout (*Oncorhynchus mykiss*) gill, has recently been adopted by OECD, marking the first in vitro-based test guideline in ecotoxicology. The assay serves as in vitro alternative to the traditional fish acute toxicity test, the most commonly used animal test in environmental risk assessment. We here present the development of the RTgill-W1 cell line assay, discuss its advantages and limitations, and illustrate examples of application. The gill cell line was selected to reflect the presumed primary target organ in acute exposure scenarios with its damage causing fish death. Cells are used in confluent monolayers, reflecting the gill epithelium. Exposure is done in a completely defined exposure medium, L-15/ex, for 24 h in 24-well tissue culture plates. Chemical concentrations in the exposure medium are quantified at the onset and upon termination of exposure. Cell viability is analyzed by three fluorescent indicator dyes, measured on the same set of cells, and compared to unexposed cells, which serve as control. Combining the cell viability measurements and the measured chemical exposure concentrations, effective concentrations impacting 50 % of the gill cells (EC50 value) are calculated based on concentration-response modelling. These values are taken at face value to predict acute fish toxicity. One 24-well plate is required per one test chemical and biological replicate.The RTgill-W1 cell line assay has been extensively validated using a wide range of chemicals with different physico-chemical properties, modes of toxic action and toxicity, overall providing excellent forecasts of fish acute toxicity. Specifically acting neurotoxic chemicals, however, appear less sensitive in the RTgill-W1 cell line assay compared to fish. As well, if indications are available that transformation products might be of relevance to toxicity, performing the test as well with that transformation product is recommended. Applications of this assay are manifold, offering a simple and resource-efficient alternative to the conventional test with fish. Chemicals of Unknown or Variable composition, Complex reaction products or Biological materials (UVCB) can be tested, including surfactants and polymers. As well, mixture toxicity of formulated products can be systematically explored. The assay is as well standardized to analyze the acute toxicity of water samples as outlined in ISO21115.

01.20.12 Pfos-Induced Developmental Neurotoxicity and Metabolomic Dysregulation in Zebrafish Embryos

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PFOS has been categorized as a persistence organic pollutant, causing various toxicities. Previous studies have been conducted to reveal molecular mechanism of PFOS toxicity; however, PFOS-induced metabolomic perturbation remains largely unknown. We investigated metabolomic alteration and identified metabolic pathways affected according to the developmental stages. Based on developmental toxicity assessment, LC_{50} and BMD_5 were determined to be 23.02 and 5.71 mg/L, respectively. Behavior analysis of zebrafish larvae was performed at sub-lethal concentrations, and metabolomics analysis was carried out at a concentration of 2.5 mg/L at 72 and 120 hpf (hours post-fertilization). Zebrafish larvae showed hyperactivity during light period and both hyper- and hypo- activity during dark period in a concentration dependent manner. We found that PFOS significantly altered the expression of metabolites and their profiling was varied by measurement time points. . Expression of L-cystathionine and 3-phosphoglyceric acid at 72 hpf, and D-serine, acetyl-L-carnitine, choline, taurine and L-pyroglutamic acid at 120 hpf were significantly different from that in the control group. Metabolic pathway analysis indicated that cysteine and methionine metabolism at 72 hpf, and taurine and hypotaurine metabolism at 120 hpf were activated by PFOS exposure, respectively. Taurine, and hypotaurine linked to earlier metabolites of cysteine and methionine, are metabolites that are known to be associated with neurological function. Glycine, serine, and threonine metabolism were enriched at both time points. Taken together these findings demonstrated that metabolomic dysregulation fluctuated with developmental time points well explains biomolecular mechanism underlying neurotoxicity of PFOS in zebrafish.

01.20.13 Potential Dermal Exposure Risk of Photodegraded Polycyclic Aromatic Hydrocarbons

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Polycyclic Aromatic Hydrocarbons (PAHs) are a diverse group of pyrogenic and petrogenic chemicals that result from wild-fires, automobile exhaust, and oil refining processes. These chemicals are ubiquitous in areas of heavy industry, and many have been reported as toxic compounds to humans by the US Environmental Protection Agency (USEPA). Although the chemical changes of PAHs under UV radiation have been recognized, the modification of toxicity to humans has not been studied. The objective of the present study is to assess the phototoxic effects of a priority PAH, anthracene, on human skin through 2D and 3D human skin keratinocyte culture models. Anthracene was degraded under simulated sunlight at 0,1,4, and 8 hours in water at 1ppm. Treatments with degraded or non-degraded anthracene products were applied to the 2D or 3D cultured keratinocytes. Overall, longer degradation time results in decreased cell viability within eight hours of photodegradation. Eight-hour degradation decreased cell viability by as much as 25.68% compared to the undegraded anthracene treatment ($p< 0.05$). GC-MS analysis confirmed that anthracene is nearly absent from solution while a group of intermediate products were generated in the eight-hour degradation. The migration of keratinocyte cells in 2D culture was also altered by photodegraded anthracene. Keratinocytes treated with the eight-hour photodegradation product demonstrated significantly enhanced migration compared to the cells treated with non-degraded anthracene for all tested concentrations. In addition, 3D cultured keratinocytes demonstrated increased stratification at eight-hour degradation treatments compared to non-degraded anthracene. These results suggest that photodegradation can alter the toxicity of PAHs in humans.

01.20.14 Protective Effects of Epigallocatechin-3-gallate (EGCG) in Arsenic-Induced Fibrogenic Changes in Human Kidney Epithelial Cells

M.S. Iheanacho, K.P. Singh, Texas Tech University / Department of Environmental Toxicology

Renal fibrosis is a histological hallmark of chronic kidney disease (CKD) and believed to be a pathogenic intermediate for CKD progression. In epidemiologic studies, arsenic exposure has been associated with adverse kidney disease outcomes including kidney fibrosis. On the other hand, epigallocatechin-3-gallate (EGCG) -a green tea polyphenol has been shown to have antioxidant, anti-inflammatory, and demethylating properties. Potential antifibrogenic effect of EGCG in kidney is not known. Therefore, the aim of this study is to determine whether EGCG can inhibit and reverse Arsenic-induced acute injury and long-term exposure associated fibrogenic changes in kidney epithelial cells. To address this question, Caki-1 cells, a human clear cell renal cell carcinoma (ccRCC) line that have epithelial characteristics, were exposed to 10ng/ml of Arsenic. For acute exposure, cells were exposed for 72hours. For long term exposure, cells were exposed and allowed to reach approximately 80% confluency. They were then sub-cultured and treated again and this cycle of treatment continued for 27 weeks. Preliminary results show that arsenic-induced acute toxicity can be prevented by EGCG. An increased production of reactive oxygen species (ROS) both in the acute and long-term Arsenic exposed cells and its decrease by EGCG was also observed . Fibronectin, a marker for fibrosis was elevated in arsenic treated cells, whereas co-treatment with EGCG down regulated this marker. The result of this study suggests that arsenic, through production of ROS, causes acute injury in acute exposure and fibrogenic changes in long-term exposure in these cells. EGCG can prevent the arsenic-induced cytotoxicity and fibrogenic changes in kidney epithelial cells.

01.20.15 Short-Chain Per- and Polyfluoroalkyl Substances (PFAS) Effects on Human Phase I Biotransformation Enzymes

M. Solan, R. Lavado, Baylor University / Environmental Science

Per- and polyfluoroalkyl substances (PFAS) are a class of compounds that have been of interest to human and ecological risk assessors in recent years because they are found ubiquitously and there is evidence for adverse health effects in humans. Newer, short-chain alternatives have been developed to mitigate some of the toxic effects observed with the long-chain variants. However, there is a significant lack of toxicity data pertaining to these compounds, and even less is known about their effects in the context of exposures with other xenobiotics. A previous study has suggested that two of the legacy PFAS, perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS), are able to reduce the expression of important CYP-enzymes in human liver cells following exposure. We evaluated the activity of two phase I biotransformation pathways (CYP1A1 and CYP3A4) following exposure to short-chain PFAS, including perfluorobutane sulfonic acid (PFBS), 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate (GenX), perfluorohexane sulfonate (PFHxS), perfluorohexanoic acid (PFHxA), 6:2 fluorotelomer alcohol (6:2 FTOH), using well-established human colorectal (Caco-2) and liver (HepaRG) cell lines. Following single exposures, each of the compounds of were co-exposed to known CYP enzyme inducers, Rifampicin and Benzo(a)pyrene, to evaluate the inhibition potential of the short-chain PFAS. Our results indicate that the interference of short-chain PFAS with CYP1A1 and CYP3A4 enzymes could potentially lead to adverse outcomes resulting from the inability of biotransformation pathways to function as needed.

01.20.16 Strategies for Adding a Data Rich Upstream Key Event to the Existing Adverse Outcome Pathway Oxidative DNA Damage Leading to Chromosomal Aberrations and Mutations””

E. Huliganga, University of Ottawa; F. Marchetti, Health Canada; J. O'Brien, Environment and Climate Change Canada / Ecotoxicology and Wildlife Health Division; V. Chauhan, Health Canada; C.L. Yauk, University of Ottawa

Adverse outcome pathways (AOPs) synthesize toxicological information from various sources to convey and weigh evidence in an accessible and transparent format. The development of AOPs requires finding the most relevant information that can be used to evaluate the weight of evidence supporting a pathway. To do this, it has been proposed that reproducible search methods, such as systematic review (SR), be used. However, applying these methods to AOP development in data-rich areas can be difficult as AOP developers are often small teams. We explored the use of a SR search structure and tools to develop a roadmap for the integration of a new key event (KE), increase in cellular reactive oxygen species (ROS), into a previously developed AOP, 'Oxidative DNA Damage Leading to Chromosomal Aberrations and Mutations'. Our research focused on developing the key event relationships (KERs), as there are numerous relevant KEs existing in the AOP-wiki. Terms relevant to ROS and DNA damage were used for a literature search to determine the extent of empirical evidence and quantitative understanding of the new KERs. These terms retrieved 235,632 papers, demonstrating that this an extensively studied pathway and that a conventional SR would not be feasible. Thus, to identify a few examples of empirical evidence to support the AOP, we sorted the papers by 'relevance' and screened the first 100. After removing irrelevant papers not containing empirical data, we determined which KER within the AOP the remaining 40 papers supported. There were 36 papers with evidence linking an increase in ROS to DNA strand breaks, suggesting strong support. In contrast, there was only 1 paper with evidence linking increases in ROS to chromosomal aberrations. This may point to the need for further empirical studies to define this KER. In this instance, the application of the SR tools was useful to establish a general level of support for this pathway and potential data gaps. However, we found that conventional use of SR tools and processes for AOP development in a data-rich area was not feasible for our team because of the extent of information retrieved. Another challenge was choosing the KE to use in the development of our proposed KERs, as there were many well-developed KEs associated with increases in ROS. Future work will determine, along with the international community, which ROS KE is preferred and approaches for applying SR strategies to identify support for KERs and define data gaps.

01.20.17 Systemic Pesticides in Japanese Orchard Bee (Osmia cornifrons) Pollen Stores Affect Larval Development and Increase Pupal Mortality

N.T. Phan, E. Rajotte, Penn State University / Entomology; N. Joshi, University of Arkansas / Department of Entomology & Plant Pathology; D. Biddinger, Pennsylvania State University / Entomology

Solitary bees, while providing pollination services, are often exposed to various pesticides applied for pest control on farmland. There is increasing evidence that sublethal toxicity of agricultural pesticides affects solitary bees differently than social bees (e.g., honey bees and bumble bees). Solitary bee studies are challenging because of the difficulties in obtaining large numbers of eggs or young larvae and due to their univoltine life cycle. Here we show the toxic and sublethal developmental effects of four widely used plant systemic pesticides on the Japanese orchard bee (*Osmia cornifrons*). Pollen stores of this solitary species were collected and treated with different concentrations (1/10X, 1X, and 10X) of three insecticides (acetamiprid, flonicamid, and sulfoxaflor) and a fungicide (dodine) based on previously measured field-realistic concentrations in apple orchard pollen. Eggs were transplanted to the treated pollen in rearing chambers and hatching larvae were allowed to feed on the pollen stores. The effects of chronic ingestion of contaminated pollen were measured until adult eclosion. This year-long study revealed that chronic exposure to all tested pesticides caused delayed larval development, lowered larval and adult body weights. Additionally, exposure to the systemic fungicide resulted in abnormal larval defecation and increased mortality at the pupal stage, indicating potential threats to bees from fungicide exposure. These findings highlighted potential new threats to solitary bees from systemic insecticides and fungicides and will be helpful in understanding and mitigating these effects.

02.01 Adapting Techniques and Approach of Standard Toxicity Test Methods to Atypical Ambient, Effluent, and Other Sample Types

02.01.01 Is That Flocculant Truly Toxic or Did the Laboratory Study Design Miss the Mark?

N. Love, GEI Consultants, Inc.

Dischargers of nearly every category throughout the world from basic domestic wastewater treatment to highly technical metals processing generally require the use of some products and chemicals to either assist in treating wastewater or to assist in other specialized processes. In many cases the products are benign and would not be expected to cause toxicity to aquatic life in the receiving stream, but in some cases, the compounds may cause toxicity if used in high doses. Prior to use of these chemicals, most dischargers evaluate the ecotoxicity of the product based on the data provided on the Safety Data Sheets (SDS) to ensure their expected dose would not lead to toxicity in the environment or failure of whole effluent toxicity (WET) tests. However, not all product SDS's are created equal and in fact, many offer very little to no ecotoxicity information. In general, there are standardized practices in place for laboratory studies generating ecotoxicity data. For example, Good Laboratory Practices (GLP) are commonly followed for ecotoxicity data collection for pesticides in the United States and Canada, but not necessarily for other types of compounds. Even when following GLP, there are many toxicity testing requirements that are not specified making study design incredibly important to ensure reliable data collection. Non-GLP labs must ensure consistency and reliability when determining the definitive ecotoxicity of a product. Numerous challenges are encountered with the use of a-typical compounds in toxicity testing. One challenge is that the simulation of a product used in processing or in a treatment system can be difficult to achieve in a laboratory setting. Many of these products have an adherent consistency, making it difficult to ensure exact dosages are measured and administered properly. In addition, standard analytical methods to measure the exact concentrations of the product that were tested in each dose for verification purposes are unavailable for a-typical compounds which can lead to an over or underestimation of toxicity in the final effluent. Another important challenge includes the introduction of highly toxic, proprietary products to a clean, uncontaminated laboratory and the possibility of propagating any residual toxicity through the continued use of exposed labware. This session will further discuss these challenges and considerations when designing studies to assess chemical and product toxicity tests.

02.01.02 Novel Approach to Identifying Toxic Ion Compounds

A. Romero, GEI Consultants, Inc. / Aquatic Toxicology Laboratory; M.S. Bowersox, Tetra Tech, Inc. / Lab

Assessing toxicity related to multiple ions and ionic balance is exceedingly difficult. Standardized assessment approaches do not exist, are limited in their descriptions and methodology or may be dependent on one ion being the causative toxicant. GEI Consultants Inc. (GEI), and Tetra Tech (TT) conducted specialized studies in attempts to identify specific ions, or the overall imbalance of ions, in effluent that were contributing to periodic chronic sublethal, or reproductive, effects to the *Ceriodaphnia dubia* in a discharge. Initially, ion-related toxicity was identified and to further narrow down a specific ionic parameter, continued investigations involving additional manipulations of the effluent outside of the standard USEPA Tier I and II protocol were conducted. The investigations involved innovative approaches using synthetic mock effluent testing, targeted ion composition modification through barium treatment, and ion exchange resins. However, numerous confounding factors were presented requiring a shift in the way the *C. dubia* tests were conducted. While TIE testing allows for flexibility in test protocol, potential shifts in protocol are not defined. The various approaches, the challenges faced within each approach, the innovative protocol design, and the outcomes of the testing will be discussed.

02.01.03 Evaluation of the Carbon-Sequestering Mineral Olivine for Potential Ecotoxicological Effects to Model Marine Organisms in Water and Sediment

P. Arth, A. Cibor, Enthalpy Analytical / Aquatic Toxicology

Bioassay methods are valuable for evaluating the potential toxicity of newly developed compounds, a finished product or individual components, and existing compounds adapting to changing applications, standards, and scrutiny. Adapting standardized toxicity test methods for novel uses and uncommon sample types requires deep scientific understanding of these tools, their appropriate uses, and their limitations. In one such example, the olivine group of minerals has shown potential to fight climate change and ocean acidification due to its rapid weathering and ability to capture and sequester carbon dioxide. Prior to undertaking large scale *in situ* pilot testing involving application of olivine minerals in marine environments, assessment of potential olivine toxicity to model marine organisms in water and sediment was considered. A series of bioassay studies were undertaken to begin this unique and complex evaluation process, and as with many novel toxicity investigations, our approach was iterative. Initial considerations included appropriate method and species selection, inherent mineral properties (such as potential for leaching of metals and physical organism interference), application rates, and other confounding factors. The first phase of investigation employed existing EPA methodology with supplemental guidance from OECD and ASTM methods and focused on evaluating potential acute and chronic effects to sediment-dwelling species including polychaetes and amphipods. The next phase is focused on vertebrate, invertebrate, and algal species in an aqueous testing environment to assess the potential of the olivine to cause toxicity to those model species. Subsequent phases of the project include potential long term exposures including bioaccumulation and additional testing with ecologically relevant species in target geographies identified for olivine deployment. The goal of the project is to produce data that can be used to assess ecologically safe application rates of the olivine in receiving environments.

02.01.04 Experimental Considerations for Assessing Coagulation Agents and Polymers for Wastewater Treatment of Coal Combustion Products

W.L. Goodfellow, M. Fleming, A. Steele, K.J. Kulacki, Exponent / Ecological and Biological Sciences

Coal-powered utilities' compliance with the Federal Coal Combustion Residual (CCR) Rule requires the closure or retrofit of facilities that do not meet the Rule's technical criteria for the structural integrity of surface impoundments, or that cause exceedances of groundwater protection standards. The principal closure options are closure-in-place and closure-by-removal; both require dewatering of the surface impoundment. Often as part of the dewatering process of the ash basins, coagulation agents (e.g., ferric chloride or emulsified and dry polymer agents) are used as part of wastewater treatment. The use of bench-level tests or pilot systems to determine the amount of agent necessary to achieve sufficient treatment often focus solely on meeting the wastewater treatment objectives from the chemistry perspective, which can lead to overdosing of agent as part of treatment operation. In these instances, coagulation agent or polymer may be present in excess of remaining dissolved solids available to react with the agent or polymer. For facilities that have whole effluent toxicity (WET) limits in their National Pollutant Discharge Elimination System (NPDES) permits, this may lead to potential compliance issues, as the unreacted agent or polymer can be toxic to aquatic organisms. WET tests using the water flea, *Ceriodaphnia dubia*, are very sensitive to overdosing of these chemicals as part of wastewater treatment. This presentation provides considerations for assessing coagulation agents and polymers as part of the selection of wastewater treatment management controls, including discussion and recommendations for designing experiments for determining potential toxicity of coagulation agents and emulsified and dry polymer agents.

02.01.05 Use of a Multispecies Algal Assay to Assess the Short-Term Aquatic Toxicity of Atrazine, Copper and Metolachlor

S. Stone, University of Technology Sydney / School of Chemistry; D.J. Koppel, Curtin University / Faculty of Science and Engineering; M. Binet, CSIRO Land and Water; S.L. Simpson, CSIRO Land and Water / Centre for Environmental Contaminants Research; D.F. Jolley, CSIRO Land and Water

In agricultural areas, the application of pesticides often results in pulsed, intermittent, and short-term exposure to pesticide chemicals by non-target aquatic organisms that inhabit waterways receiving wet-weather runoff. Understanding how variations in pesticide exposure regimes (individually and as contaminant mixtures) leads to adverse effects on organisms is important to improving the management of their use. The duration and timing of exposure to contaminants can have a significant impact on observed toxicity to aquatic organisms; however, predicting the risk of toxicity from these exposures remains a significant challenge as water quality guideline values are mostly based on data from continuous exposures. Atrazine, metolachlor, and metals such as copper are common herbicide contaminants known to be highly toxic to freshwater microalgal species. Our research examines some knowledge gaps in risk assessments posed by short-term exposure to contaminants by investigating the pulse toxicity of these contaminants. We examined how changing exposure duration altered their toxicity using a multispecies microalgal assay with three tropical, freshwater species: *Monoraphidium arcuatum*, *Nannochloropsis*-like sp., and *Pediastrum duplex*. Toxicity was assessed individually, and as binary and tertiary mixtures under four exposure scenarios: continuous 72-h exposure, 18-h pulse exposure and 3-h pulse exposures in either the photosynthesis (in the light) or the cell division (in the dark) phases. Exposure duration had a significant effect on toxicity for all three species, with microalgae tolerating up to 14 times higher concentrations of herbicides as individual contaminants in 18-h pulses, compared to continuous 72-h exposures. Atrazine did not inhibit algal growth following a 3-h pulse. Metolachlor and copper toxicities following the 3-h pulse exposures were dependent on cell metabolism at the time (i.e. if they were dividing or photosynthesizing). We investigated whether continuous toxicity data could effectively predict mixture toxicity under varying exposure scenarios. These data highlight the need to consider the exposure duration and exposure photoperiod in toxicity testing to underpin the regulation of herbicides in aquatic ecosystems.

02.01.06 Characterizing Variability Among Environmental Toxicology Laboratories in Canada With Proficiency Testing

L.A. Van der Vliet, R.P. Scroggins, Environment and Climate Change Canada / Biological Assessment and Standardization Section

Accreditation of environmental toxicity laboratories in Canada is linked to the international standard ISO/IEC:17025. As part of national accreditation, environmental testing laboratories are required to participate in proficiency testing (PT, also called proficiency evaluation). For PT tests in chemistry, microbiology and toxicology, a common sample is prepared and circulated to all participating laboratories at set intervals throughout the year. Laboratories treat the PT samples as routine samples, and report their toxicity results to the PT provider and the accreditation body. In Canada, most toxicology laboratories use the Canadian Association for Laboratory Accreditation (CALA) as their accreditation body. If the PT provider and/or CALA deems that the performance on PT evaluation is unacceptable, the laboratory must, at a minimum, complete a corrective action report, and submit it to CALA for approval. Repeated PT failures will prompt additional actions by CALA, such as suspension of accreditation. Proficiency testing functions well as a routine, third-party evaluation of the ability of laboratories to consistently measure the toxicity of a common sample. Rainbow Trout and *Daphnia magna* acute lethality tests have high regulatory relevance in Canada, as test results are routinely used in federal effluent regulations and provincial permitting programs. For these two tests, many laboratories in Canada use the same proficiency test provider, PT Canada. Aggregated and coded toxicology laboratory results for about 20 participating laboratories are available from the PT Canada website for each 6 month round of testing. Although the primary purpose of PT is as a quality assurance mechanism, what can these results show us about variability between laboratories? How does this compare to variability within a specific lab? Can we make meaningful comparisons of variability between PT results for environmental toxicology and PT results for chemistry measurements? What are the limitations with repurposing PT as a way to estimate variability between laboratories? Does PT isolate for biological variability of the test organism? When faced with the decision to deem one or more PT results "unacceptable", would you make the same decision as the accreditation body? This presentation will explore these questions, using real proficiency testing data collected in Canada.

02.01.07 New Aquatic Toxicity Testing Guidance Document Brings Clarity to Ecotoxicology Testing of Nanomaterials

E. Petersen, National Institute of Standards and Technology / Biosystems and Biomaterials Division; G. Goss, University of Alberta / Biological Sciences; A. Kennedy, U.S. Army Engineer Research & Development Center / Environmental Laboratory

Manufactured nanomaterials (MNs) may have novel or enhanced properties compared to bulk materials with the same elemental composition, leading to a wide range of potential commercial applications. With the potential for widespread usage of MNs in consumer products, it is essential to reproducibly assess their potential for ecological and human health risks during a product's life cycle. However, one of the early questions in nanotechnology environmental health and safety research was to what extent currently available standard methods can be used to assess the ecotoxicological risks of MNs. To fulfill this need, international scientists worked together from 2014 to 2020 to develop specific guidance to apply OECD aquatic toxicity test methods for water column organisms (e.g., algae, fish, *Daphnia*), with additional considerations relevant to sediment toxicity testing, for use with MNs. This resulted in the recent release in July 2020 of OECD guidance document 317 titled "Guidance document on aquatic and sediment toxicological testing of nanomaterials." In this presentation, we will discuss the primary findings and recommendations from this guidance document on key topics such as MN suspension methods, dosimetry, modifications to test media to support MN stability, and potential control experiments to avoid experimental artifacts.

02.01.08 Water Quality Monitoring and Ecological Risk Assessment of the Veldwachters River, Cape Town

S. Mlonyeni, Cape Peninsula University of Technology / Department of Environmental and Occupational Studies; O.K. Pereao, B. Opeolu, Cape Peninsula University of Technology / Environmental Toxicology and Chemistry Research Group Faculty of Applied Sciences

The significance of a river for ecosystem function in a climate-changing world is increasingly important. This is because rivers provide humans with socio-economic service functions that predispose them to increasing exploitation, degradation, and pollution. The Veldwachters River is a non-perennial river, which is recharged with the effluent discharge from a wastewater treatment plant (WWTP) that receives domestic and municipal wastewater. This study investigated the seasonal variation of the river's physico-chemical characteristics. The acute toxicity potential of the effluent being discharged into the Veldwachters River was also evaluated. A battery of tests was used to assess ecotoxicological effects of effluent discharge on the river. *Daphnia magna* (primary consumer), *Raphidocelis subcapitata* (primary producer) and *Tetrahymena thermophila* (decomposer) were used as model organisms. The values for pH, dissolved oxygen (DO), electrical conductivity (EC), temperature, redox potential (ORP) and total dissolved solids (TDS) levels were in the range of 4.7 – 9.7, 2.4 – 7.6 mg/L, 612 – 701 µS/cm, 18.7 – 28.4 °C, 41 – 220 mV and 405 – 470 mg/L, respectively. Acute toxicity test of effluent on *D.*

magna showed 5% mortality of daphnids. The *R. subcapitata* exposure resulted in biomass growth and the yield increased after 48 h and 72 h. The ecotoxicity results suggested that the effluent exhibited slight acute toxicity to freshwater organisms. Although the effluent contributes positively to the river recharge and health downstream, it has the potential to trigger eutrophication in the river system. The results provide insights into the benefits and possible risks that must be mitigated by governments and WWTP authorities for reuse and optimal utilization of freshwater resources for sustainability.

02.01.09 Episodic Exposures - Modified Toxicity Test Method Application Considerations for NPDES Compliance Monitoring and Risk Assessment

C. Stransky, Wood Environment & Infrastructure Solutions, Inc. / Aquatic Sciences and Toxicology; M. Colvin, NIWC Pacific (Naval Information Warfare Center Pacific) / Energy and Environmental Sustainability; N. Hayman, Naval Information Warfare Center Pacific / Energy & Environmental Sustainability Branch; G.H. Rosen, SPAWAR Systems Center San Diego / Energy and Environmental Sustainability

Understanding the potential ecological impacts from episodic discharges to receiving water bodies is a significant challenge and a rapidly growing area of research. Many permittees across the nation are required to monitor and comply with various water quality objectives for stormwater runoff and other episodic discharges. These requirements generally include end-of-pipe monitoring, enforced by National Pollutant Discharge Elimination System (NPDES) permits, prior to mixing in the receiving water. Regulatory concern with stormwater discharges is associated with the Clean Water Act's goal to prevent discharge of toxics in toxic amounts. As a result, the existing EPA whole effluent toxicity (WET) test methods developed to assess continuous point source discharges are now being applied to episodic discharges as well. A four year ESTP-funded study is nearing completion that has developed and validated a more realistic toxicity test method for episodic discharges such as stormwater to provide a more environmentally-relevant and accurate assessment of the potential for receiving water impacts. This method follows existing EPA WET guidance with a simple modification to the exposure method and timing. This presentation will highlight a proposed decision framework where the modified toxicity test exposure method may be most appropriately considered and applied for both regulatory compliance monitoring and as an additional tool for traditional risk assessment. Case study examples will be provided for studies conducted in both marine and freshwater environments.

02.01.10 Optimizing Sex Ratios of Hyalella azteca to Assess Reproductive Toxicity

H. Khan, Y. Kudla, R.S. Prosser, University of Guelph / School of Environmental Sciences; A.J. Bartlett, Environment and Climate Change Canada / Water Science and Technology Directorate

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. Standard toxicological test methods for this species incorporate both lethal and sub-lethal (growth, reproduction) endpoints, though lethal endpoints are often favoured when testing in the context of environmental monitoring. However, sub-lethal endpoints are important to consider as they are ecologically relevant and are often more sensitive than lethality. It can be 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. Furthermore, males of the species partake in complex interactions when they compete for mates which adds to the variability in brood sizes that females produce. The purpose of this study was to characterize the reproductive capacity of *H. azteca* by determining the role of sex ratios in reproductive yield. It was hypothesized that a lower male: female ratio will reduce intraspecific male aggression, improve reproductive success, and lower biological variability in the total number of young produced. Experiments were initiated in the absence of toxicants with 6-7-week-old individuals that were placed in different male to female ratios (1:1, 2:3, 3:2 and 3:7). Reproduction was monitored weekly for 7 weeks to determine which sex ratio had the least variable reproductive output over time. Preliminary results suggested that reduced reproductive variability occurred in treatments with low male to female ratios. The results from this research will provide insight into optimizing the reproductive capacity of *H. azteca,* which will increase the ability of reproductive toxicity tests to capture the effect of chemicals or contaminated sediment on amphipod reproduction.

02.01.11 Application of Fundulus heteroclitus Health Indicators to Establish Baseline Environmental Quality of the Anacostia River

A. Vazquez, University of Maryland, College Park / ENST; A.E. Pinkney, U.S. Fish and Wildlife Service / Chesapeake Bay Field Office; L.T. Yonkos, University of Maryland / Environmental Science and Technology

The Anacostia River is one of the more heavily contaminated tributaries of the Chesapeake Bay, and is a manifestation of environmental inequity in the capital of the United States. The legacy of urban and industrial pollution continues to harm aquatic biota and imperil disadvantaged residents who fish the tidal waters for subsistence. Establishing baseline toxicological conditions within the Anacostia is necessary to monitor the effectiveness of large-scale remediation efforts planned for the next decade. We are using the mummichog, *Fundulus heteroclitus*, to study fish health at sites along the tidal portion of the river associated with historical sources of persistent organic pollutants (e.g., PAHs, PCBs, OC-pesticides). Mummichog are a practical fish model due to their abundance in the tidal Anacostia, small home range (< 500m), short life span (3-4 yr.), sexual dimorphism, close association with benthic sediments, and predictable spawning behavior. The species is also relevant to the food web, serving as common prey and bait for sport fish. We are investigating a suite of mummichog toxicological and reproductive health indices including: tissue contaminant concentrations; embryo-larval development and survival upon sediment exposure; histopathology of adult liver, spleen and ovary; Gonadosomatic index; and fecundity and oocyte quality across the spawning season. Results will serve as a benchmark of current Anacostia environmental quality for comparison during and after remediation. As the Anacostia is characteristic of other urban rivers, lessons learned will be applicable to similarly degraded systems and especially germane to regions where rapid urbanization and substantial poverty ensure significant reliance on locally-caught fish as a food source.

02.01.12 Challenges Associated With Interpreting Reverse Osmosis Whole Effluent Toxicity Testing Results for Freshwater and Marine Discharges

M.K. Chanov, EA Engineering, Science, and Technology, Inc., PBC / Ecotoxicology; J. Redifer, EA Engineering, Science, Technology, Inc., PBC; R. Brooks, EA Engineering, Science, and Technology, Inc., PBC / Toxicology Lab; M. Durbano, EA Engineering, Science, and Technology, Inc., PBC

The increased efficacy of wastewater treatment has resulted in the removal of the majority of classical toxicants in industrial wastewaters. However, as a result, the concentrations of total dissolved solids (TDS) in industrial wastewaters has become increasingly important. Some facilities have resorted to the use of reverse osmosis systems as a wastewater treatment technology to deal with these effluents. However, the use of reverse osmosis can create issues with reverse osmosis reject water disposal and the discharge of ion deficient effluents. The discharge of both types of effluent can inadvertently create a similar pattern of toxicity when compared to the initial effluent. This presentation discusses cases studies for both freshwater and marine discharges that illustrate the challenges associated with these complex wastewaters and the adaptive strategies that need to be used to address the toxicity associated with these types of effluents. These case studies evaluated the toxicity of the facility's effluent, using standardized acute and chronic whole effluent toxicity (WET) testing. Following the confirmation of toxicity through the WET testing, the samples were further evaluated utilizing combinations of standardized and innovative toxicity identification evaluation (TIE) procedures to identify the dominant toxicants. These case studies indicate that the regulatory approach and considerations differ substantially for freshwater and marine discharges, which confounds the overall approach for dealing with these discharges.

02.01.13 Interlaboratory Calibration Comparisons for Pulsed Exposure Toxicity Testing

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Episodic discharges (e.g. stormwater, dry-dock discharges, and pesticide applications, etc.) require environmentally-relevant, scientifically-defensible, and conservative toxicity test designs to assess potential for receiving water impacts. Currently, permittees in highly industrialized areas are regularly required to conduct 96-hour (or longer) toxicity tests on discharges associated with events that are often less than 24 hours in duration. Existing EPA whole effluent toxicity (WET) test methods developed to assess continuous point source discharges are now being applied to episodic discharges as well. However, these methods do not adequately reflect episodic discharge conditions at either the point of compliance (i.e. storm drain) or as it mixes with the receiving environment (e.g. a riverine or marine system), which can result in an overestimation of toxicity at a given site. In order to capture representative toxicity at a site, an alternative toxicity test approach is described, incorporating pulsed exposures to end-of-pipe samples. Following pulsed exposures, organisms are transferred to uncontaminated seawater (or receiving water) for the remainder of standard test period. This presentation presents the results of an Interlaboratory Calibration Study conducted in order to assess the ability of the modified methodology to provide consistent and defensible data for the assessment of episodic discharges. The study characterized 1) Completion Rate; 2) False Positive Rate; and 3) Precision on three WET methods that were modified for pulsed exposures and included acute tests with *Ceriodaphnia dubia* and *Americamysis bahia* and the larval development/short-term chronic test using *Strongylocentrotus purpuratus*.

02.01.14 Microbiological Analysis for Accelerated Degradation by Increasing the Medium Volume in Ready Biodegradability Test

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A ready biodegradability test is required in many countries to evaluate the environmental fate of chemical substances. The Organisation for Economic Co-operation and Development (OECD) 301C test has been conducted under the Japanese Chemical Substances Control Law (CSCL), in which the volume of test medium is specified to 300 mL. In addition, the OECD 301F test has also been approved under the CSCL since 2018, in which the medium volume is not specified. Takekoshi *et al*. found that degradation of 2-ethylanthraquinone (2-EA) or tris(2-ethylhexyl) trimellitate adsorbed on silica gel was accelerated by increase of the medium volume under the conditions that concentrations of chemical, silica gel and activated sludge were held constant. However, the reason for the accelerated degradation has not been directly clarified. In this study, metagenomic analysis of microorganisms in the sludge was conducted to investigate the reason of the accelerated degradation. The 301F tests were conducted for dibenzofuran and 2-EA by changing volume of the test medium from 300 mL to 3900 mL under the conditions that concentrations of the test chemical and activated sludge were kept at 100 and 30 mg L^{-1}, respectively. Dibenzofuran was directly added into the test medium, while 2-EA absorbed on silica gel was added into the test medium. Concentration of the silica gel in the test medium was fixed to 1333 mg L^{-1}. Microbial DNA of the activated sludge was extracted from the test medium when the test substance was biodegraded. The 16S rRNA gene (V3-V4 region) of the extracted DNA was subjected to metagenomic analysis using a next-generation sequencer. As a result, biodegradation of both the chemicals was accelerated by increasing the test medium, and microorganisms species that could biodegrade the chemical increased as the medium volume increased. It was considered that increasing the test volume accelerated biodegradation of chemical substance by securing various microorganisms in the test medium.

02.02 Advancing the OMICS Into Regulatory Frameworks: Case-Studies and Perspectives

02.02.01 Evaluating the Temporal Stability of Gene Expression Endpoints with Repeated Sampling Up and Down Stream of a Wastewater Treatment Plant

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Transcriptomic endpoints are increasingly being used in environmental toxicology and monitoring. Laboratory-based studies generally are highly controlled and aim to evaluate a select number of chemicals or conditions, limiting the effects of confounding factors. This makes it possible to directly link the experimental condition with the response and greatly simplifies interpretation of transcriptomic responses. In contrast, interpretation of field-based studies is complicated by the temporal dynamics of source inputs, water quality, and considerable unknowns in chemical exposures. While previous research used transcriptomic endpoints to evaluate potential deleterious effects or as biomarkers of contaminant exposures in aquatic environments, few (if any) such research sought to characterize the stability of global gene expression in field applications. Our study aimed to characterize the stability of gene expression in three commonly used applications: (1) source characterization, (2) exposure biomarkers, and (3) effect characterization. Gene expression was compared between five treatments of matched groups of exposed larval fathead minnow (FHM; Pimephales promelas) exposed to water upstream from and within the effluent of a wastewater treatment plant. Four treatments consisted of grab samples taken in the morning and afternoon over two consecutive days and brought back for static exposures under controlled ambient conditions. The fifth treatment consisted of in situ exposed organisms deployed at the same site and time as the grab samples. RNA sequencing analysis was conducted on whole FHM larvae. Statistical enrichment of overlap among time points and exposure treatments were identified by examining the multi-set intersections of differentially expressed transcripts versus a combinatorial-based null model. Classifiers were developed for each time-matched upstream and downstream pair using several common classification algorithms which were tested on their ability to classify other time-matched pairs and the in situ exposure. To evaluate consistency in the biological response, functional enrichment was also conducted for each of the time-matched pairs. This analysis demonstrates the robustness and stability of gene expression biomarkers in common application and exposure scenarios and may provide important insight into the limits of interpretation of transcriptomic endpoints.

02.02.02 Investigating Variability in Transcriptomics-Based Points of Departure for Fish - Investigating the Effects of Exposure Vessel for Copper and Sertraline

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Before a chemical is sold for commercial use, toxicity testing is required to evaluate possible environmental impacts. Traditional toxicity tests have several limitations, overlooking important subtle effects as well as costing time and resources. A proposed solution, transcriptomic points of departure (tPODs) have been gaining traction in environmental toxicology as a possible metric for use in environmental regulation. While several studies have succeeded in finding transcriptomic points of departure that are protective of traditional apical endpoints, there is a notable lack of studies that aim to characterize variability of transcriptomic points of departure. One important aspect of a study that could introduce variability is exposure vessel. Different studies use different exposure vessels which may introduce minor factors resulting in observed variation. Using different vessels can affect the chemical concentration through different evaporation rates or interactions with vessel walls. In addition, differences in the amount of water and swimming space may impact stress of the organism leading to altered gene expression. The current study utilized three different exposure vessels, 96-well plates, 24-well plates, and 6-well cups for two chemicals with distinct modes of action, copper sulfate and sertraline. Larval fathead minnows (*P. promelas*; 5-6 days post-fertilization) were exposed to either copper sulfate or sertraline for 24 hours in one of the exposure vessels with 8 doses and 6 biological replicates per dose. RNA was isolated from each whole organism and sequenced. Sequence data was used to calculate tPODs that were compared. The results of this study will shed light on variability introduced by different exposure vessels commonly used in toxicology testing. While variability will always exist, it is necessary to minimize as much as possible with the purpose of effectively comparing data across multiple tests and developing meaningful environmental policy.

02.02.03 Using OMICS to Assess the Neurotoxicity of Bifenthrin to Salmonids

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An increase in urban and agricultural application of pyrethroid insecticides in the San Francisco Bay Estuary and Sacramento San Joaquin Delta has raised concern for the populations of several salmonids, including steelhead trout and Chinook salmon. Bifenthrin, a type I pyrethroid, is among the most frequently detected pyrethroids in the Bay-Delta watershed, with surface water concentrations often exceeding chronic toxicity thresholds for several invertebrate and fish species. Since the brain is considered to be a significant target for bifenthrin toxicity, and previously shown to induce behavioral changes in Chinook when co-exposed to increased water temperature, juvenile steelhead trout, rainbow trout, and Chinook salmon were exposed to concentrations of bifenthrin previously measured within the Delta with subsequent evaluations of metabolomic and transcriptomic profiles and histopathological effects assessed within this target. Pathway analysis software predicted induced cellular apoptotic and necrotic responses in brains of steelhead exposed to 60 ng/L bifenthrin, which was largely driven by a reduction of acetyl-L-carnitine, docosahexaenoic acid, and adenine. Brains of steelhead exposed to 120 ng/L bifenthrin had reductions of lysophosphatidylcholines, lysophosphatidylethanolamines, and increased levels of betaine, which were linked to inflammatory responses. Metabolomic profiles similarly predicted increased apoptotic, inflammatory, and reactive oxygen species responses in brains of Chinook following exposure to 0.15 and 1.50 µg/L bifenthrin. These responses were largely driven by reduced levels of inosine, hypoxanthine, and guanosine. Transcriptomic profiling determined that the top bioinformatic pathways predicted to be impaired in the brains of rainbow trout treated with 15 and 30 ng/L bifenthrin were involved in gonadotropin releasing hormone signaling, dysregulation of iron homeostasis, reduced extracellular matrix stability and adhesion, and cell death. Subsequent histopathological assessment showed significantly increased TUNEL-positive cells in the cerebellum and optic tectum of bifenthrin-treated rainbow trout. These data indicate that bifenthrin may have multiple targets within the brain that affect general neuron viability, function, structural integrity, and signaling.

02.02.04 Assessing the Effects of Dietary Exposure to PPAR Agonists in Mummichogs (Fundulus heteroclitus)

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Evidence has grown that some persistent compounds found in the environment may have impacts on growth, reproduction, and metabolic homeostasis in mammals and fish that are mediated in part by interaction with the peroxisome proliferator-activated receptor (PPAR) pathway. To better understand the effects PPAR perturbation may have on populations of wild organisms, we exposed the estuarine fish *Fundulus heteroclitus* to the polybrominated diphenyl ether BDE99 and the human therapeutic clofibric acid (CLA) in diet. Although data are limited, both compounds interact with the PPARα pathway in fish and serve as model compounds for PPAR dysregulation. In the current study, wild-caught adult mummichog were individually tagged and placed in tanks receiving flowing seawater at 23 °C. Fish in four replicate tanks/treatment (2 male, 2 female/tank) were fed diets amended with acetone (control), BDE99 (75, 150, 375, or 750 ng/g fish ww/day), or CLA (80 µg/g fish ww/day) for 38 days. Weight and length were monitored throughout, and breeding was assessed by manual strip-spawning. On days 10 and 39, half of the fish were euthanized, and fish weight, length, and wet weight of gonad, liver, abdominal fat, and brain were recorded. Energetic reserves, as represented by changes in abdominal fat and liver mass, were affected in a dose-responsive manner by day 39. At day 10, fish in all BDE99 treatments were reproductive, but by day 39 all BDE- and CLA-treated fish exhibited dramatically reduced egg production compared to controls. Perturbations in the hepatic metabolome were also dose responsive, albeit more pronounced in females at the later timepoint after exposure to BDE99 and CLA. Assessment of transcriptomic, proteomic, and lipidomic responses and bioaccumulation is ongoing and will support a multi-omic approach to elucidate PPAR-mediated molecular toxicity pathways in fish. The results will also be extrapolated ecologically using bioenergetic and population models developed for this species. This work will support the development of Adverse Outcome Pathways and provide insight into outcomes of exposures to emerging contaminants of concern that are proposed to interact with PPAR in ecological species.

02.03 Alternative Approaches to Animal Testing for Ecotoxicity Assessments: Exploring Approaches and Avenues for the Future

02.03.01 An Integrated In Vivo and In Silico Framework to Study the Physiological Consequences of Targeted Metabolism Disruption in Embryo-Larval Zebrafish (Danio rerio)

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Integrating the functions of large-scale metabolic networks with physiological measurements (such as omics-based high-throughput datasets and O_2 consumption rate) can help us to develop a quantitative framework with which to study organismal metabolic physiology. Furthermore, such an approach can help to predict the impact of metabolic disruption (by pollutants, metabolic/endocrine disrupters) on regulatory-relevant apical endpoints that are reflective of organismal fitness (such as survival, growth, etc.). This presentation describes the integration of *in vivo* experimental data with an *in silico* stoichiometric metabolism model of zebrafish, to study the metabolic pathways perturbed under exposure to the mitochondrial metabolism disrupter, CPI-613, which inhibits tricarboxylic acid (TCA) cycle activity. Embryo-larval life stages of zebrafish (*Danio rerio*) were exposed to 1 µM CPI-613 for 20 days. Whole-organism respirometry measurements showed an initial suppression of O_2 consumption at Day 5 of exposure, followed by recovery comparable to the solvent control (0.01% DMSO) by Day 20. Comparison of whole-transcriptome RNA-sequencing at Day 5 vs. 20 of exposure showed functional categories related to O_2 binding and transport, antioxidant activity, FAD binding, and hemoglobin complexes, to be commonly represented. Metabolic enzyme gene expression changes and O_2 consumption rate was used to parametrize two *in silico* stoichiometric metabolic models representative of Day 5 or 20 of exposure respectively. Computational simulations predicted impaired ATP synthesis, α-ketoglutarate dehydrogenase (KGDH) activity, and fatty acid β-oxidation at Day 5 vs. 20 of exposure. These results show that the targeted disruption of KGDH may also impact oxidative phosphorylation (ATP synthesis) and fatty acid metabolism (β-oxidation), in turn influencing cellular bioenergetics and the *in vivo* observed reduction in whole-organism O_2 consumption rate. The results of this study provide an integrated *in vivo* and *in silico* framework to study the impacts of metabolic disruption on organismal physiology. The methods presented may be of value for toxicological studies that integrate ‘big data’ omics datasets with targeted physiological measurements (such oxygen consumption rate).

02.03.02 Application of In Vitro Mass Balance Models to Facilitate the Interpretation and Use of In Vitro Toxicity Data for Hazard and Risk Assessment

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In vitro toxicity data using fish and mammalian cell lines are increasingly common in the peer-reviewed literature but their use for quantitative risk assessment is challenged by the frequent use of nominal medium concentrations to report the dose-response curve. The main objective of this presentation is to introduce and demonstrate the utility of an updated, high-throughput mass balance model for predicting the distribution of organic chemicals in in vitro test systems (IV-MBM EQP v2.0). The model has been implemented as a publicly available Excel/VBA tool and as an online interactive version (www.eas-e-suite.com). The IV-MBM EQP v2.0 tool was first parameterized and applied to four in vitro test data sets using algae, fish or human cell lines with measured ratios of bulk medium or freely-dissolved to initial nominal concentrations (e.g., C24/C0 where C24 is the measured concentration after 24 hours of exposure and C0 is the initial nominal concentration). Model performance varied depending on the data set, chemical properties (e.g., “volatiles” vs. “non-volatiles”, neutral vs. ionizable organics) and model assumptions but overall is deemed acceptable. For “non-volatile” neutral organics included, the r^2 was greater than 0.8 and the mean absolute error (MAE) in the predictions less than a factor of two. Model performance was not as good for the ionizable organic chemicals included but the r^2 was still greater than 0.7 and the MAE approximately a factor of two. When reported, the model was used to “translate” effects data (i.e., nominal EC50s, AC50s) to a predicted membrane concentration and compared to a threshold for baseline toxicity (i.e., >20–60 mM). The IV-MBM EQP v2.0 tool was subsequently applied to several hundred chemicals on Canada’s Domestic Substances List (DSL) with AC50s reported for five representative ToxCast assays. The nominal AC50s corresponded to predicted membrane concentrations in the baseline toxicity range for approximately 35-50% of the chemicals. All chemicals can exert baseline toxicity as a toxic Mode of Action (MOA) and so effects data in this range from in vitro toxicity testing may be a true reflection of MOA. However, it may also be an indication of a lack of sensitivity in the in vitro assay. A combination of in vivo, in silico and in vitro assessments of MOA is therefore recommended for hazard assessment. The use of IV-MBM EQP 2.0 is also recommended for risk assessment purposes (i.e., “dose translation”).

02.03.03 Assessment of Toxicity-Normalized Species Sensitivity Distributions (SSDn) for Grouped Chemical Hazard Estimation

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New approach methods are being developed to address the challenges of reducing animal testing and assessing risks to the diversity of species in aquatic environments with minimal data. The toxicity-normalized species sensitivity distribution (SSDn) approach is a novel method for developing compound-specific hazard concentrations using data for toxicologically similar chemicals. This approach first develops a SSDn composed of acute toxicity values for multiple related chemicals that have been normalized by the sensitivity of a common species tested for each chemical. A toxicity-normalized hazard quotient (HC5n) is then computed from the 5^{th} percentile of the SSDn. Chemical-specific HC5 values are determined by back-calculating with the HC5n and the chemical-specific toxicity data for the normalization species. The accuracy and uncertainty of the SSDn approach was evaluated for nine transition metals and compared to HC5s derived for the individual metals using conventional single chemical SSDs. We identified several guiding principles for this method that, when applied, resulted in highly accurate HC5 values based on comparisons with results from single metal SSDs. The SSDn approach shows promise for developing statistically robust hazard concentrations when adequate taxonomic representation is not available for a single chemical.

02.03.04 An In Vitro Tier Based Approach to Predict Acute Whole Effluent Toxicity in Fish

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The RTgill-W1 cell line assay (ISO 21115:2019), and the Fish Embryo Test (FET; ISO 15088:2007 and OECD 236:2013) have shown to be effective in vitro methods to predict toxicity of waterborne chemicals to fish in vivo. Applying these methods to acute Whole Effluent Toxicity (WET) testing allows a better understanding of toxicological impacts at the cellular and early life stage level, respectively. The development of an in vitro tiered WET testing approach may offer an alternative to current in vivo methods as well as a more sensitive and specific toxicity forensic tool (e.g. for Toxicity Identification/Reduction Evaluations strategies). However, considerations of the practicality of in vitro approaches must also be made to better understand their role towards advancing WET testing methods.

In this study we examined 20 wastewater and effluent samples, and 10 matrix spiked effluent exposure scenarios and evaluated their toxicity in side-by-side experiments in RTgill-W1 cells (24 h), the fathead minnow (*Pimephales promelas*) FET (96 h) and the fathead minnow larvae (96 h). Toxicity was observed for 14 samples for the FET, 24 samples for RTgill-W1 cells, and 21 samples for larvae. Overall, RTgill-W1 cells successfully predicted toxicity to fish larvae of all actual wastewater samples. Additionally, we investigated if effluent osmotic manipulations (i.e. comparing hypoosmotic (150 mOSm/kg) and isosmotic conditions (300 mOSm/kg)), could improve the sensitivity of RTgill-W1 cells. Out of the 5 osmotically manipulated samples, results indicated that RTgill-W1 cells were able to effectively predict toxicity for 3 samples in isosmotic, and for all 5 samples in hypoosmotic conditions. Furthermore, RTgill-W1 cells were successfully incorporated as a TIE/TRE tool to facilitate identification of specific classes of chemicals for the observed toxic effluents using subcellular endpoints (i.e. morphology, metabolic activity, membrane and lysosomal integrity). Based on our findings, RTgill-W1 cells are able to predict acute toxicity in WET samples and could be used for identification of toxicants. Moreover, if incorporated as a tier-based strategy, RTgill-W1 cells can serve as the first point of toxicity confirmation, reducing the need of using live fish for acute WET testing.

02.03.05 Incorporating the 3Rs Into the Development of a Standardized Test Method Using a Native Amphibian Species

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The assessment of aquatic toxicity is an integral part of environmental risk assessment of chemicals, and has therefore been included in important environmental and chemical regulations and legislation around the world. While there is growing demand for standardized tests using native amphibian species to inform risk assessment, tests with a mortality endpoint using vertebrates raise ethical concerns. Environment and Climate Change Canada (ECCC) has incorporated replacement, reduction, and refinement strategies during the development of its standardized test method for assessing contaminants with a native amphibian species (*Lithobates pipiens*, Northern leopard frog). Two test options are available depending on research objectives: a 14-day test beginning with Gosner stage 25 tadpoles that covers a sensitive growth period, useful for assessing general toxicity; and a 42-day test beginning with Gosner stage 28 or 29 tadpoles that covers a portion of metamorphosis, useful for assessing changes in developmental rate, as well as growth, over a longer exposure period. Data from the literature and ECCC's Atlantic Laboratory for Environmental Testing facility support the use of rainbow trout acute toxicity data (96-hour LC50) as a surrogate or replacement for larval amphibians such as *L. pipiens*, therefore only chronic toxicity test procedures will be published for this species. High priority was given to reducing the number of test organisms used per experiment by optimizing test design using power analysis, maximizing the number of test endpoints that can be obtained from a single experiment, and using a positive control instead of a multi-concentration reference toxicant test. Lastly, the Canadian Council on Animal Care is a long-standing institution that provides advice on refinement for all vertebrate testing, and the reach of on-site animal care committees has grown substantially in recent years. To emphasize the importance of these groups without duplicating effort, the ECCC standardized test method will refer to published guidance documents and local authorities for refinement practices with amphibians.

02.03.06 Determining Mode of Action of Substances in Ecological Risk Assessments Under Canada's Chemicals Management Plan

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The mode of action (MoA) of a substance can be defined as the manner in which a substance causes an adverse effect due to critical physiological changes in the cell upon interaction with biological macromolecules (such as lipids, proteins or nucleic acids). MoA is increasingly recognized as a key line of evidence for informing ecological hazard and risk assessment of substances. Consideration of MoA can assist with the selection of appropriate predictive models, analogue justification, and to inform life-stage or species-specific susceptibility. Mode of action can also be a key consideration in the selection of appropriate assessment factors (AFs) used for deriving predicted no-effect concentrations. In a novel AF selection method developed by Environment and Climate Change Canada (ECCC), substances suspected of exhibiting a narcotic ecotoxicological MoA are assigned a lower AF compared to those that are suspected of having a non-narcotic MoA (e.g., reactive, specifically acting). However, empirical data on MoA are often lacking, including for most substances undergoing risk assessment under Canada's Chemicals Management Plan (CMP). To determine whether a substance has a narcotic or non-narcotic ecotoxicological MoA, ECCC and Health Canada have developed a systematic framework that can be practicably applied in risk assessment. This framework relies on a weight of evidence approach based on the output of multiple predictive tools, including *in silico* tools, calculations of critical body residue and lethal activity, with comparison to known effect levels for baseline narcosis. This approach for MoA differentiation, as well as case studies from CMP substance assessments, will be presented.

02.03.07 Eliminating Water Controls in Fish Toxicity Studies That Use Solvents: How Can Agreement Among All Stakeholders Be Reached?

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A team led by PETA Science Consortium International e.V. and the United States FDA, with collaborators from industry, CROs, academia, research institutions and regulatory agencies in the US, Europe and Japan, is working on a project to determine whether only a solvent control is required in addition to treatment groups in aquatic fish studies that use a solvent. A successful project would reduce the number of fish used and the costs of regulatory studies. Scientific Issues: The main issue is whether this can be done without compromising the ability to detect or estimate effects of importance to regulatory risk assessment from guideline studies. We need to determine to what extent the answer to the main question depends on the specific type of studies (e.g., acute or chronic fish studies), life stage, endpoints (e.g., survival only, growth and reproduction), purpose (e.g., standard toxicity endpoints or endocrine disruption specific), species, and solvents. Regulatory Acceptance Issues: How much and what type of data, and what modelling and analysis techniques are sufficient to justify regulatory authorities to accept a recommendation to use only the solvent control in studies when a solvent must be used? Plan of Attack: To date the analysis has focused on OECD TG 203, the fish acute toxicity study with mortality as its major endpoint, and OECD TG 210, the Fish Early Life Stage study with survival and development (length and weight) as major endpoints. In each effort a database of guideline studies has been collected and studied. This raises the question of how large a database is required to warrant a change in the regulatory requirements? Would a detailed analysis of one fish species and one solvent be enough? Would data from only 2 or 3 labs be adequate? Would a focus on the key endpoints from each type of study be sufficient or should all potential endpoints be included? The goal is to obtain a global standard on the types of fish studies where only a solvent control is needed when a solvent is used rather than putting the onus on individual labs to justify omitting the water control in specific types of studies. The presentation will provide a brief update on the progress that has already been made and provide more information on the regulatory challenges. We also invite ideas and data from the SETAC community.

02.03.08 Quantum-Mechanical Models and Their Integration Into Computational Frameworks for Design of Commercial Chemicals with Minimal Ecotoxicity

J. Kostal, A.M. Voutchkova, P. Griffin, J. Lewer, The George Washington University / Chemistry

Designing safer chemicals is a multifaceted challenge that requires simultaneous consideration of metrics such as synthetic feasibility, performance, toxicity and environmental-fate. By large, this analysis cannot be done effectively using experimental models; for example, the ethical and economical costs of animal models to comprehensively assess PBT (persistence, bioaccumulation and toxicity) are too prohibitive for bulk chemicals. Yet, ignoring these criteria in the upstream of new product development can be detrimental, leading to high downstream costs of chemical production and commercialization. Over the past 10 years, a diverse portfolio of cost-effective *in silico* models has emerged to address this need. These tools range from structure-based to system-based models, from small dataset-derived mechanistic approaches to 'big' data-driven machine learning methods; some are used to predict PBT outcomes, some environmental fate or transport, and others tackle technical challenges related to chemical synthesis. Some survive real-world scrutiny and become (commercially) successful; others fail but often pollinate future development efforts. By large, however, these tools are developed in a vacuum, lacking systems-based thinking that is essential to holistic design of future safer chemicals. Here, we examine how several quantum-mechanical tools, developed and validated to accurately predict acute and chronic aquatic toxicity, can be integrated into broader computational frameworks that also consider chemical performance and environmental fate. Two exemplary chemical classes are discussed: pesticides, where we intersect metrics of ecotoxicity, photodegradation and function, and ionic liquids, where we identify chemical space that maximizes performance in biomass deconstruction while minimizing hazard to aquatic species. Our aim is to promote crosstalk between niche *in silico* solutions to stimulate future efforts that consider broader integration strategies in the initial development of predictive tools. From a risk and hazard assessment perspective, this is a vital step toward successful replacement of traditional testing methods with New Approach Methodologies (NAMs).

02.03.09 Enhancing Marine Pollution Effects Monitoring Through New Targeted Environmental DNA (eDNA) Testing in the Pacific Northwest

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Globally, coastal waters experience degradation from pollution associated with multiple discharges including storm water, industrial and agricultural runoff, and nutrient enrichment from municipal wastewater. The responses of benthic communities to nutrient enrichment and often associated low-oxygen conditions has some striking parallels worldwide. For example, certain indicator taxa, particularly certain polychaete species, are well-known to proliferate in response to high nutrient input and corresponding anoxic conditions while others, such as some echinoderms and amphipods, are sensitive to these conditions. Traditional assessment of macroinfaunal benthos involves the detailed analysis of each individual specimen within a sample by taxonomic experts. This activity is labour intensive, requiring both the meticulous removal of organisms from the sample debris, and their identification by highly trained personnel. Damage to some organisms incurred during sampling at substantial depths often makes taxonomic identification very difficult or impossible. Our research aims to develop powerful and sensitive environmental DNA (eDNA) assays to detect these indicator species in an efficient and reliable way to assess organic enrichment. eDNA is extra-organismal DNA that can be isolated from environmental samples such as a scoop of water or sediment. Using whole genome sequencing we have generated mitogenome sequences for multiple benthic indicator species routinely used for monitoring programs in Pacific Northwest marine environments. We developed a pipeline for identifying unique DNA sequences conducive for robust quantitative polymerase chain reaction (qPCR) assay development. Using these new assays, we are evaluating multiple field collection and sample processing protocols to enable faster and accurate benthic biota identification and quantitation. These methods are conducive to standardization and wide-scale adoption of eDNA sampling into marine environmental effects monitoring in the Pacific Northwest.

02.03.10 Adverse Outcome Pathway (AOP) Guided High-Throughput In Vitro and In Vivo Assessment of Mitochondrial Toxicants

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Mitochondria are the energy warehouses in eukaryotes. A wide range of chemicals can affect mitochondrial energetic functions, leading to adverse effects of regulatory concern (e.g., growth inhibition and reproductive failure). By aligning the concepts of adverse outcome pathways (AOP), new approach methodologies (NAMs) and animal alternatives (AAs), this study aims to develop a tiered testing strategy for cost-efficient hazard assessment of mitochondrial toxicants. A suite of high-throughput *in vitro* assays using the ZF-L liver cell-line and *in vivo* assays using zebrafish embryos have been developed, as guided by an established AOP linking uncoupling of oxidative phosphorylation (OXPHOS), reduction of ATP pool, reduction of cell proliferation and growth inhibition (OECD project #1.92, AOPWiki, AOP #263, https://aopwiki.org/aops/263). In the next step, the zebrafish cells and embryos will be exposed to known mitochondrial uncouplers to generate temporal and concentration-response data. The responses will be compared between the *in vitro* and *in vivo* testing systems for the three early key events (KEs) in the AOP. Chemical analysis will also be performed to support *in vitro* to *in vivo* extrapolation (IVIVE). Results obtained from these analyses will suggest whether the *in vitro* assays can replace the *in vivo* tests, or whether the two testing systems can be better incorporated to form a tiered testing strategy.
This study was funded by the Norwegian Research Council (project 301397, www.niva.no/en/projectweb/riskaop) and supported by the NIVA Computational toxicology Program (www.niva.no/en/projectweb/nctp).

02.03.11 Cytotoxicity and mRNA Expressions of Five Bisphenol a Replacement Compounds in Primary Chicken Embryonic Hepatocytes and LMH 3D Spheroids

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A market for bisphenol A (BPA) replacement compounds has emerged due to restrictions on the use of BPA. Primary chicken embryonic hepatocytes (CEH) are commonly used for *in vitro* avian toxicity testing. Previously, we showed that the immortalized chicken hepatic cell line, LMH, grown as 3D spheroids, may be a suitable animal free alternative to CEH for chemical screening. In this study, we evaluated the utility of LMH for identifying and comparing potential endocrine disrupting chemicals (EDCs). Cytotoxicity and mRNA expression were evaluated following exposure of CEH and LMH 3D spheroids to BPA, five replacement compounds (BPF, TGSH, DD-70, BPAF, BPSIP), and 17β estradiol (E2). Cytotoxicity and gene expression were determined using ATP concentrations and two customized PCR arrays, respectively. The ToxChip array contains genes from toxicologically relevant pathways and the AestroChip array measures estrogen-responsive genes. DD-70 and BPAF were the most cytotoxic replacement compounds in both CEH and LMH 3D spheroids. TGSH and DD-70 modulated the greatest number of genes among replacements and BPA on the ToxChip array in both models, although fewer genes were altered in LMH spheroids. Based on the gene expression profile of E2, BPF was the most estrogenic in CEH, while BPAF was the most estrogenic in LMH spheroids.The cytotoxicity and clustering of the replacements based on gene expression profiles were similar between CEH and LMH spheroids; however, LMH spheroids were more sensitive in terms of estrogenic response. In addition to generating novel gene expression data for five BPA replacement compounds in an *in vitro* avian model, this research also demonstrates that LMH spheroids may represent a useful animal free avian model for identifying EDCs.

02.03.12 Aquatic Toxicity Characterization and Prioritization of E-Cigarette E-Liquid Chemicals

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The increase in the use and sale of electronic nicotine delivery systems (ENDS) raises concerns about their potential environmental impacts. The U.S. Food and Drug Administration (FDA) is required to assess the environmental impacts of its tobacco regulatory actions as mandated by the National Environmental Policy Act (NEPA). To assess potential environmental impacts, identification of the specific chemicals and their ecotoxicological hazard characteristics are needed, but such information for ENDS products is currently limited. The purpose of this study was to identify commonly used ENDS chemical constituents, characterize their aquatic toxicity hazard potential, and to identify chemicals of concern for impacts on aquatic organisms. A total of 342 unique chemical constituents in ENDS e-liquids were compiled and identified from peer-reviewed literature and e-liquid database (ingredient database developed by University of North Carolina). The aquatic toxicity of these chemicals was characterized through hazardous concentration values (HC; concentration affecting 50% of aquatic species; HC_{50}) and Globally Harmonized System of Classification and Labelling of Chemicals (GHS), along with the bioaccumulation factors of the chemicals. Aquatic toxicity endpoint data were predicted through quantitative structure-activity relationships for chemicals without adequate ecotoxicity data and the predicted values were utilized to estimate HCs. Among the chemicals identified, acrolein, allyl hexanoate, beta-pinene, nicotine and metals (copper, cadmium, uranium and zinc) were the most hazardous ENDS constituents for acute aquatic toxicity (injurious to an organism in a short-term aquatic exposure). For chronic aquatic toxicity (injurious to an aquatic organism with long-term effects), squalene, methyl ester octadecanoic acid, methyl ester hexadecanoic acid, beta-pinene, benzyl benzoate and metals (copper, cadmium, and uranium) were the most hazardous ENDS constituents. Sixty-five of the 342 ENDS chemicals were very toxic or toxic for acute and chronic aquatic toxicity based on GHS. These results provide a comprehensive identification and characterization of the ecological hazards posed by commonly used ENDS chemical constituents. This study also identifies chemicals of potential concern, aiding prioritization of compounds for regulatory review and overall evaluation of the environmental impacts of ENDS products for NEPA.

02.03.13 Toxicity Evaluation of Parabens and Their Halogenated Wastewater Disinfection Byproducts Using In Vitro Human and Fish Cell-Based Models

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Parabens is the name given to a group of p-hydroxybenzoic acid (p-HBA) esters used in over 22,000 cosmetics as preservatives. Their safety is well documented in mammalian models, but little is known about their toxicity and metabolism in non-mammalian models. Chlorinated and brominated parabens as a result of water treatment have been identified in wastewater effluent. In this study, we explored the cytotoxic effects (LC50) of the most commonly used parabens (methyl-, ethyl-, propyl-, butyl-, and benzylparaben) and the primary metabolite, 4-hydroxybenzoic acid as well as 3 of the wastewater disinfection byproducts (methyl-3 chloro-4-hydroxybenzoate, methyl-3,5 dichloro-4-hydroxybenzoate, and methyl-3,5-dibromo-4-hydroxybenzoate) on fish cell lines from different tissues (catfish and rainbow trout gills; Japanese eel, rainbow trout, and fathead minnows hepatocytes) and human cell lines from different origin too (hepatocytes and enterocytes). Intrinsic clearance rates of the parent parabens were also established using S9 fractions from the same cell lines. LC50 values for the parent parabens demonstrated several orders of magnitude difference between fish and human cells, suggesting the fish may be more susceptible to toxic effects. A strong relationship was observed between toxicity and the increase in chain length of the parent paraben. All the disinfection byproducts tested showed higher toxicity than the parent parabens, but the fish cell-based models were more susceptible.

02.03.16 Developing a New Approach to Assess Crop Protection Chemical Safety that Minimizes Reliance on Vertebrate Testing and Protects Human Health and the Environment

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The USEPA intent to eliminate reliance on vertebrate tests from 2035 challenges the research-led Crop Protection (CP) companies to develop new approaches in order to meet the regulatory requirements for new Active Ingredients (AI), as no guidance currently exists to meet the EPA's goal whilst ensuring no unreasonable risk to human health and the environment. To determine the scope and direction of a project, Syngenta and USEPA OPP senior leadership and scientific staff held a problem formulation discussion resulting in a draft problem statement: "Establish a scientifically sound strategy that applies appropriate and flexible exposure and effects characterization without chemical-specific vertebrate tests to address the risk assessment needs that provides confidence in regulatory decisions". To resolve this problem, we set to partner with USEPA on a project where data generated according to the current data requirements and test guidelines, as well as the new approaches can be submitted and reviewed in parallel for the purpose of new AI registration. The project objectives are to create a worked example applying existing conceptual frameworks based on modern scientific approaches to identify and characterize the dose-range over which potential adverse effects may occur relative to the anticipated exposure from proposed uses of a new AI, determine whether these frameworks meet the USEPA's risk assessment needs for human health and vertebrate ecotoxicology, and to identify where further development of new approaches for compound-specific data generation would be required. To maximise the reuse of existing data, we selected a new herbicidal AI with an established mode of action having many exemplars for which the toxicology and ecotoxicology is well characterized. We demonstrated that these data can be curated and analysed to provide an appropriate human health and vertebrate ecological hazard characterisation for the purpose of risk assessment of such new AI. We further explored its possible extension to CP chemicals with less extensive existing exemplar datasets and highlight the identified uncertainties and gaps that will require additional new approaches.

02.03.17 Developmental Outcomes and Gene Expression Profiling Reveals Early Toxicological Mechanisms of Lead Effects in an Early-Life Stage Amphibian, Xenopus laevis

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The environmental distribution of lead is widespread, mainly as a result of anthropogenic activities. Reported environmental concentrations of lead can elicit a wide variety of adverse effects in amphibians, including mortality, developmental abnormalities, endocrine disruption and altered behaviour. Toxicogenomic responses to lead exposure could help identify specific molecular mechanisms that drive the adverse effects of lead and thus be used to predict apical outcomes of ecological and regulatory relevance for amphibians. The objective of this research was to determine the effects of early life stage exposure to lead on *Xenopus laevis* (XL) tadpoles and characterise the early toxicological mechanisms of lead toxicity in amphibians. Embryos were exposed at 48 hours post-fertilization to 70, 210, or 630 µg/L lead nitrate ($Pb(NO_3)_2$) in quintuplicates, and exposures were continued for three weeks. Individuals were evaluated for mortality, growth, development, and the incidence/severity of developmental abnormalities. Measured concentrations of lead in exposure water over the three-week exposure were 78-90% of nominal and while there was no significant mortality in any of the lead-exposed groups, tadpoles in the high lead group (630 µg/L) had reduced total body length and increased incidence (46.3%) of developmental abnormalities. A subset of individuals (5 individuals pooled per replicate) from each exposure group was sampled at 96 h and whole-body transcriptome profiles were assessed using high throughput RNA sequencing (RNASeq). Raw files were analyzed using alignment-based (Hisat2) and pseudo-alignment (Kallisto) workflows in the EcoToxXplorer-hosted Galaxy server to obtain the raw counts. Differential gene expression analysis was performed using the XL-specific gene expression modules in www.ecotoxxplorer.ca and revealed 328 significantly dysregulated genes including those related to DNA repair, cell cycle, apoptosis, immune function and metabolism. Targeted gene expression analysis will also be conducted using a XL EcoToxChip, a species-specific gene expression array developed as a rapid and economic tool for chemical risk assessment and management that supports alternatives to animal testing by using early life stage organisms. Overall, the results of the present study will provide insight into the developmental effects of lead exposure and underlying mechanisms of lead toxicity on early life-stage tadpoles. This study is part of the EcoToxChip project (www.ecotoxchip.ca).

02.04 Assessing Contaminant Effects in Ecosystems with Multiple Stressors

02.04.01 Environmental and Anthropogenic Factors Affecting Rivers of the Chesapeake Bay Watershed with Implications for Land Management

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If not managed properly, urban development and agricultural practices can alter surface-water and groundwater quality, including in drinking water resources, resulting in potential negative effects on aquatic and terrestrial ecosystems. Exposure to contaminant mixtures has the potential to alter habitat quality and negatively affect fish and other aquatic organisms. The Chesapeake Bay watershed provides many benefits, including contributions to the economy, recreational activities such as sportfishing, drinking water for millions of people, and habitat for numerous fish and wildlife species. For more than a decade, fish species in the Chesapeake Bay watershed have exhibited skin lesions and observed estrogenic endocrine disruption, which has raised concerns for fish reproduction and survival. Several studies have focused on understanding the occurrence, effects, and potential risk of contaminant mixtures and other environmental drivers on fish health throughout the watershed. Results from these studies have increased our understanding of the 1) spatiotemporal variation in organic contaminants in surface and groundwater, 2) drivers of contaminant exposures, 3) influence of land-use on contaminant occurrence and potential risk and 4) benefits of land management strategies in reducing contaminant concentrations and improving fish health. These and other studies continue to inform future priorities related to water and habitat quality, fill important data gaps, inform management priorities, and contribute to a better understanding of the presence, relationships, drivers, and effects of contaminants and other stressors within the Chesapeake Bay ecosystem.

02.04.02 Impacts of Glyphosate on T- Lymphocyte Proliferation in Florida Manatees

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The immune system's role is to protect against infectious disease and exposure to chemicals can suppress immune processes allowing their development. Glyphosate is the most used herbicide worldwide and it has been found in Florida waterbodies chronically exposing the Florida manatee. In Florida, it is used as a ripener in sugarcane, to control weeds, before and after harvest. It can also be sprayed to control invasive aquatic plants, among other uses. The objective of this study was to analyze the consequences of exposure to glyphosate and a commercial formulation in the Florida manatee adaptive immune response via T-cell proliferation. Proliferation assays were performed using lymphocytes isolated from blood samples of 12 free-ranging animals in December-January 2018 and December 2019. Each healthy manatee had complete blood cell count and total protein quantification in serum. Lymphocytes were exposed to 0, 10, 1000, and 10,000 µg/L of glyphosate and to equivalent concentrations of glyphosate in Rodeo® and simultaneously to a mitogen (phytohemagglutinin 5 µg/mL) at a concentration of 100,000 cells/well in triplicate. The proliferation capacity was significantly affected by the treatments and sampling period. Glyphosate caused a dose-dependent reduction in the proliferation capacity of lymphocytes with a significant decrease at 10,000 µg/L of glyphosate with a mean reduction of 72%. The effect of glyphosate on Florida manatee's immune system had a more pronounced effect in some individuals with a reduction of the proliferation capacity in relation to the effect of the mitogen by itself of down to 56% at 10,000 µg/L and 67% at 1,000 µg/L of glyphosate. The glyphosate formulation did not produce a more pronounced effect than the same concentration of glyphosate. The proliferation of T-lymphocytes was significantly affected by the sampling period with animals sampled in January having reduced proliferation capacity when compared to manatees sampled in December. After one month of cold weather, healthy manatees in January had a higher concentration of white blood cells, heterophils, platelet numbers, and total protein in serum that likely affected their T- cell proliferation capacity. Our findings should be considered in the context of a threatened species that is already facing other environmental stressors that affect their immune response such as red tide and cold stress.

02.04.03 Salinities Influence on Bioaccumulation and Sublethal Toxicity to Pesticides in a Model Estuarine Organism (Menidia beryllina)

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Rising sea levels and changes in precipitation patterns are linked with changes in salinity in some estuaries. Recent studies have shown that the toxicity of some chemicals may change depending on the exposure salinity. Other studies have also shown that the bioaccumulation factor (BCF) can change in seawater compared to fresh. As such, the differences in toxicity and bioaccumulation of compounds across a salinity gradient is a topic of increasing interest in assessing risk to estuarine fish species. Therefore, we are conducting a bioaccumulation study and sublethal toxicity testing on the Inland Silverside (*Menidia Beryllina*), a model, estuarine fish species at 5 PSU and 25 PSU to seven different commonly used pesticides: bifenthrin, chlorpyrifos, dicloran, myclobutanil, paraquat, penconazole, and triadimefon. Previous studies with these compounds showed significant differences in mortality to triadimefon between 5 and 15 PSU. Differences in behavioral toxicity to bifenthrin at different salinities have been seen in other studies as well. Current exposure concentrations will be set at the compound's LC25 values at 5PSU salinity which have been determined in a previous study. Bioaccumulation will be measured in *Menidia* embryos from less than 24 hours post fertilization to one day pre-hatch (6 days in total). Sublethal toxicity exposures will include developmental, behavioral, and gene expression endpoints and last from less than 24 hours post fertilization to 4 days post hatch (12 days in total). Results will assess differences in embryo bioaccumulation and larval behavior and development based upon the hypothesize that there will be differences in bioaccumulation and sublethal toxicity endpoints at the different salinities, with bioaccumulation and toxicity increasing as salinity increases. This investigation will provide knowledge to risk assessors and managers of estuarine and marine habitats as data on chemical toxicity across salinity gradients are lacking.

02.04.04 Biological Assemblages Degraded by Multiple Instream Stressors Across Five Large Regions in U.S.

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Biological assemblages in streams are affected by a wide variety of physical and chemical stressors associated with land-use development, yet the importance of combinations of different types of stressors is not well known. From 2013–2017, the U.S. Geological Survey completed multi-stressor/multi-assemblage stream ecological assessments in five regions of the United States (434 streams total). Diatom, invertebrate, and fish communities were enumerated, and five types of potential stressors were quantified: habitat disturbance, excess nutrients, high flows, basic water quality, and contaminants in water and sediment. Boosted regression tree (BRT) models for each biological assemblage and region generally included variables from all five stressor types and multiple stressors types in each model was the norm. Classification and regression tree (CART) models then were used to determine thresholds for each BRT model variable above which there appeared to be adverse effects (multi-metric index (MMI) models only). In every region and assemblage there was a significant inverse relation between the MMI and the number of stressors exerting potentially adverse effects. The number of elevated instream stressors often varied substantially for a given level of land-use development and the number of elevated stressors was a better predictor of biological condition than was development. Using the adverse effects-levels that were developed based on the BRT model results, 68% of the streams had two or more stressors with potentially adverse effects and 35% had four or more. Our results indicate that relatively small increases in the number of stressors of different types can have a large effect on a stream ecosystem.

02.04.05 The Development of a Risk and Decision-Making Framework to Manage the Multiple Stressors and Endpoints of the Upper San Francisco Estuary

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We are conducting a program to build a Bayesian network relative risk model to estimate risk and then apply an adaptive management process to the Upper San Francisco Estuary 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. A major issue has been the organizing of the 250,000 entry dataset that comprises observations from 2010- 2019, does not have duplication, and with each entry georeferenced. We are now driving two roads to estimating risk. One is the use of the dataset to establish relationships between macroinvertebrate structure with water quality data and contaminant exposure. The details of this are described in a complimentary report by E. Lawrence. The second road is the use of the dataset to estimate the risk to the fish and water quality endpoints. 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. Tools such as case-learning are being applied to construct the cause effect pathways based on the latest understanding of the Bayesian network relative risk model. A major finding is the challenge of constructing datasets built of decades, integrating them with toxicity information in the form of regressions, and connecting them to the patterns of the biotic and water quality endpoints of the analysis. We will provide examples of how this effort connects multiple stressors into risk estimates.

02.04.07 Comparing Contaminant-Induced Gene Expression in Native and Non-Native Oysters in Southern California Estuaries

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Oysters provide numerous ecosystem services that benefit estuarine habitats including water filtration and shoreline protection. *Ostrea lurida* are the only native oyster species on the west coast of the United States but populations are significantly reduced due to historic overharvesting and urbanization of estuaries across its native range. Stressors include metal and organic contaminants and the presence of the nonindigenous Pacific oyster (*Crassostrea gigas*) that can reduce native populations due to competition for food and space. Moreover, studies suggest that *C. gigas* are more tolerant to adverse environmental conditions providing an advantage over native species. These confounding factors require immediate assessment to address the challenges facing *O. lurida* populations. The current study aimed to evaluate the differential stress response of *O. lurida* and *C. gigas* in four highly polluted Southern California estuaries utilizing high-throughput RNA-sequencing. Additionally, RNA sequencing data will be compared to sediment and oyster tissue contaminant data to assess whether molecular responses align with different types of contaminant burdens. Of the contaminants evaluated, metal concentrations varied across all sites with copper, for example, displaying increased levels in both *C. gigas* and *O. lurida* from Upper Newport Bay (UNB) and decreased levels from Agua Hedionda Lagoon. Organic contaminants (PCBs, PAHs, and organochlorine pesticides) also varied across sites with oysters from San Diego Bay near a navy operational site displaying increased levels of PCBs and oysters from UNB displaying increased levels of organochlorine pesticides. Currently, sequencing data of both species are being evaluated for differential gene expression (DEG) across populations and machine learning will be used to determine if DEG overlaps with differences in contaminant burdens. This work will further our understanding of stress response in oysters under varying contaminant burdens and address signs of stress tolerance in *C. gigas* to aid in management of a nonindigenous species.

02.04.08 Resistome Characterization of Extended-Spectrum Beta-Lactamase Positive Escherichia coli in Oregon Wastewater Treatment Plants

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Bacterial resistance to last resort beta-lactam antibiotics is an emerging threat to human health. Pathogenic *Escherichia coli* confer resistance via extended-spectrum beta-lactamases (ESBLs) encoded on plasmids (i.e., mobile genetic elements) that can easily spread via horizontal gene transfer. As junctions of clinical and domestic sewage, wastewater treatment plants collect antibiotic-resistant bacteria, accumulate their determinant genes, and facilitate their spread into the environment. Prior to this study, there has been no knowledge on the ecology of antibiotic resistance determinants ("the resistome") in Oregonian wastewater treatment plants or clinical settings . Thirteen ESBL-producing *E. coli* were isolated from 9 wastewater treatment facilities across Oregon, from wastewater influents ($n = 3$), secondary effluents (i.e., after biological treatment and before disinfection; $n = 2$), treated effluents (i.e., after disinfection; $n = 4$), and treated biosolids ($n = 4$) over winter 2019 and summer 2020. Their genomes were sequenced and characterized, identifying plasmids and other mobile genetic elements, and antibiotic resistance and virulence genes. Phylogenetic analyses revealed associations between resistance genotypes, including the potential for horizontal transfer of ESBL genes. This is the first study to report on the characterization of beta-lactam antibiotic resistance genes proliferating in the Oregonian wastewater treatment system.

02.04.09 Development of Resistance to Insecticides in Hyalella azteca

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Streams, estuaries, and other bodies of freshwater are contaminated with a variety of different insecticides due to runoff from agricultural and urban landscapes. Currently, *Hyalella azteca*, a non-target epibenthic amphipod, have developed resistance to pyrethroid insecticides due to single amino acid mutations in the voltage gated sodium channel gene. The degree of resistance in *H. azteca* is similar to that observed in several target species, including *Musca domestica*, *Cimex lectularius*, *Blattella germanica*, and *Rhipicephalus microplus*. Yet, the costs associated with the development of resistance include a loss of fitness with reduced reproductive rates, decreased thermal tolerance, and decreased tolerance to salinity. Aquatic systems are often contaminated with several different types of insecticides, therefore there is a possibility that *H. azteca* have also developed resistance to other classes of insecticides. The aim of this study is to determine if pyrethroid-resistant *H. azteca* have developed resistance to other insecticides. Three lab-cultured *H. azteca* populations (a clade C wild-type population, a clade C pyrethroid-resistant population, and a clade D pyrethroid-resistant populations) were exposed individually to the insecticides bifenthrin (pyrethroid), chlorpyrifos (organophosphate), carbaryl (carbamate), DDT (organochlorine), fipronil (phenylpyrazole), and imidacloprid (neonicotinoid) through a series of 96-h water-only acute toxicity tests. It is hypothesized that pyrethroid-resistant *H. azteca* have developed resistance to other insecticide classes due to previous long-term exposure, which makes them susceptible to cross-resistance. The development of resistance to additional insecticide classes may suggest additional population-levels vulnerabilities in field *H. azteca*.

02.04.10 The Silence of the Clams: The Effects of Environmentally Relevant Concentrations of Forestry Use Pesticides on Adult Soft Shell Clams

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The US forestry industry commonly applies an array of pesticides to control plant and insect pests. A recent study confirmed the presence of these pesticides in water as well as the tissues of various bivalve species in Oregon coastal watersheds. Though studies have been carried out to determine the individual effects of these compounds on organisms and the environment in which they live to establish lethal limits, environmentally relevant concentrations of these chemicals in combination have not been tested for sub-lethal effects. We conducted laboratory experiments to examine the effects of four commonly used forestry pesticides; Atrazine, Hexazinone, Indaziflam, and Bifenthrin, on the soft shell clam *Mya arenaria*, a common estuary species. Growth, feeding rates, and condition index were measured, as well as mortality. Final results show a significant amount of mortality in comparison to the controls, as well as observable trends in condition index and feeding rates, providing a basis for further research.

02.04.11 Effects of Early Life Stage Zebrafish (Danio rerio) Exposure to River Water Contaminated by Agricultural Activities on Physiological Endpoints

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In aquatic ecosystems, fish spawn in waters that are contaminated with hundreds, if not thousands of environmental chemicals. Exposure to these complex environmental mixtures may have important consequences on offspring, particularly early life stage (ELS) fish that are known to be sensitive to environmental contaminants. Here we evaluated how exposure to pesticide-contaminated river water affected ELS fish, subsequent development stages, and next generations. Water samples were collected in June 2019, during the fish spawning season, from the Richelieu River (QC, Canada) which runs through highly agricultural areas. Two surface water samples were collected; one upstream (Chambly) and one downstream (Saint-Ours) of tributaries fed by agricultural drainage. Nine targeted pesticides (5 herbicides, 3 neonicotinoid insecticides, 1 degradation product) previously identified in the Richelieu in 2018 were measured in the 2019 samples using liquid chromatography/mass spectrometry. Zebrafish embryos (3 treatments x 3 replicates; 100 embryos each) were exposed to river water (Chambly or Saint-Ours) or reconstituted hard water (control group). The exposure period lasted for the first 120 hours post fertilization; thereafter, zebrafish were raised to maturity (F0) in clean water. The exposed F0 generation was bred within treatment groups and raised to maturity to produce the F1 generation. Hatching time, survival, growth, tail coiling activity, sex ratio and reproductive output were measured in each generation. Three pesticides were detected at similar concentrations in Chambly and Saint-Ours samples: aminomethylphosphonic acid 1361 vs 1006 ng/L, atrazine 18 vs 20 ng/L and metolachlor 63 vs 50 ng/L. In contrast, imazethapyr (119 ng/L) was only detected in water from Chambly and clothianidin (243 ng/L) only in water from Saint-Ours. Chlorantraniliprole, glyphosate, imidacloprid and thiamethoxam were not detected. We found that exposure to river water caused delayed hatching when compared to controls in the exposed F0 generation and that this effect was more pronounced in the fish exposed to water from the downstream site (Saint-Ours). The river water had no significant effect on survival or growth in the F0 population at any life stage. Analysis of the other endpoints and evaluation of the impacts in the F1 generations are ongoing. The results will increase our knowledge on the effects of realistic mixtures of environmental contaminant exposure on ELS fish.

02.04.12 Impacts of Wastewater Effluent Stressors: Using Reactive Oxygen Enzymes as a Marker of Neurodegeneration in Darters (Etheostoma sp.) Found in the Grand River

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The Grand River (GR) watershed extends throughout the majority of Southern Ontario with its final outlet at Lake Erie. Along the GR, there are 30 wastewater treatment plants (WWTP) with varying degrees of filtration. Many of these WWTPs are currently unable to effectively eliminate all pharmaceutical by-products from their final release effluent, leading to measurable concentrations (ng/L – µg/L) in the surface waters, leaving aquatic species chronically exposed to these multiple stressors. Chronic exposures to mixed pharmaceuticals have been reported to impact oxidative stress, measurable through reactive oxygen species (ROS) production and the antioxidant defense response which helps reduce the toxicity of ROS produced molecules. This research focuses on the effects of WWTP effluent on four *Etheostoma* (Darter) species (rainbow darter; RBD, greenside darter; GSD, fantail darter; FTD and johnny darter; JD) endemic to the GR. Darters are excellent species to study mixed stressors effects due to their abundance, high site fidelity, species-specific habitat selection and varying tolerances to anthropogenic activity. This study determined whether any neurodegenerative effects in the brain of darter species found downstream from the effluent release point persist compared to an upstream site from the Waterloo WWTP (Ontario, Canada). Evidence for such neurodegeneration can be indicated by increased ROS enzymatic activity, damage, or changes in specific transcript markers in the downstream darter species. An initial assessment was conducted by using transcriptional and enzyme analysis of antioxidant enzymes (SOD, GPX, CAT) and an enzyme involved in serotonin synthesis (TPH). Significantly increased expression of transcript markers was mainly found in downstream GSD and JD species compared to upstream species analyzed ($p < 0.05$). Similarly, GSD and JD downstream displayed significantly increased antioxidative enzyme activity in the brains ($p < 0.05$). RBD and FTD both displayed some significant differences; however, impacts were greater in GSD and JD. Therefore, future studies should focus on species that may be more susceptible to wastewater effluent rather than species that are more tolerant, as they may not respond as readily. Using multiple closely related species would provide a more realistic and better understanding on subtle effects caused by mixed stressors in wastewater effluent.

02.04.13 In-Situ Mussel Bioassay with Juvenile Arkansas Brokenray (Lampsilis Reeveiana) in the Buffalo National River, AR

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The Buffalo National River (BUFF) runs free-flowing for 246 km through the Boston Mountains, Springfield, and Salem Plateaus in Arkansas. Currently, several regions are 303d listed as impaired for dissolved oxygen and bacteria, including the emergence of large algal blooms downstream of the confluence of the tributary Big Creek and Carver site. The BUFF's water quality database indicates that nitrogen compounds have been increasing since the 1980s. During the pandemic and study period, the river experienced higher tourism, in addition to flood events and park closures. This has elevated concern for the status of the BUFF's freshwater mussel resource and the toxic threats posed to juveniles during low flow conditions. Although laboratory assays demonstrate that juvenile mussels are sensitive to un-ionized ammonia and nitrates, in-situ experiments are needed to bridge the gap between biological endpoints measured in the laboratory and real-world effects due to exposure. We conducted an in-situ mussel study with juvenile Arkansas Brokenray (Lampsilis reeveiana) to investigate the potential adverse effects of nitrogen contaminants and other water quality factors. Mussels were deployed in two enclosure designs; mussel "silos", which primarily expose mussels to water, and sediment "W-V" boxes, which provide greater exposure to sediments. Five sites were chosen in reference to Big Creek (two above and three below). From June–November 2020 water quality, survival, growth, environmental conditions, and disturbances were monitored. At the end of the study, a subset of the mussels was evaluated based on body condition using multiple metrics. Differences between sites and enclosure types were expected due to the gradient of nutrient impacted areas along the river and interactions involving exposure design. Survival was 100% in silos and 84% in boxes. Overall, juveniles were larger in the silos and had consistently high body condition at the Carver site for both enclosure types. Total ammonia and nitrate concentrations in the silos ranged from 0.086 to 0.615 mg N/L and 0.13 to 1.21 mg/L respectively. Total nitrogen tracked the trends observed with ammonia and nitrate overtime. These findings provide suggestions on conducting mussel in-situ experiments in a heavily trafficked river, in addition to supporting the BUFF's long-term watershed management and the conservation of its resident mussel populations.

02.04.14 Alterations to Cd and BaP Uptake During Short-Term Waterborne Co-Exposures in Adult Zebrafish (Danio rerio)

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Cadmium (Cd) is a severely toxic trace metal commonly released as a by-product of mining. The steady increase in anthropogenic activities over the last century has resulted in Cd being an ubiquitous aquatic pollutant. While not as persistent in aquatic environments, polycyclic aromatic hydrocarbons (PAHs), a family of organic pollutants released during the incomplete combustion of organic matter and fossil fuel use, are also of great concern for aquatic organisms due to their toxicity at relatively low doses. Presently the interactive effects of Cd and PAHs on the uptake, accumulation and toxicity in fish is poorly understood. Benzo-a-pyrene (BaP), often considered as a prototypical PAH, is of particular concern due to its relatively high toxicity and is often used to assess such interactions. We have previously demonstrated that acute (72hrs) aqueous co-exposures of adult zebrafish (*Danio rerio*) to either 0.44 or 1.06 µg/L and 5.8 or 22 µg/L Cd and BaP, respectively, resulted in a greater body accumulation of both toxicants compared to that during exposures to each toxicant individually at identical concentrations. To further elucidate the mechanistic basis for this interaction, adult zebrafish were first exposed to similar doses of the two toxicants (10 µg/L Cd, 1 µg/L BaP and a mixture of the two) for either 24 or 72hrs. This was followed by a brief (3 or 6hrs) exposure to a radioactive tracer (^{109}Cd or ^{14}C-labelled BaP, respectively) to assess uptake kinetics. Flux rates were calculated separately for the gills and rest-of-body. Animals pre-exposed to both 10 µg/L Cd and 1 µg/L BaP simultaneously showed a significantly higher uptake rate of ^{109}Cd into their gills, compared to the control and single-toxicant groups. This interaction appears to be time sensitive, as it was not observed in animals pre-exposed for 24hrs. Conversely, Cd-only pre-exposed fish showed a significantly higher uptake rate of ^{14}C-BaP, unlike the mixture group. The results of the present study further elucidate the interaction between Cd and BaP during aquatic co-exposures. Our findings imply that the previously observed increase in body accumulation following co-exposures is at least partially explained by an increase of the influx rate, in the case of Cd.

02.04.15 Co-Exposure to Crude Oil and Ultraviolet (UV) Radiation Induces Cataract Formation in Fishes: A Novel Endpoint of Photo-Induced Crude Oil Toxicity

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in the environment due to both natural and anthropogenic activity. Exposure to ultraviolet (UV) radiation can significantly increase the toxicity of PAHs to aquatic organisms through photo-induced toxicity. While increased mortality is a well-documented effect of photo-induced toxicity, few studies have characterized potential sublethal effects. Impaired visual function is one sublethal effect that may greatly impact fitness and ecological performance. In fishes, the eyes are particularly vulnerable to contaminant exposure which can induce cataract formation and impair vision. The present study developed a novel method to quantify cataract formation in fish lenses following PAH exposure by measuring changes in lens absorbance and optical density. In addition, fixed wavelength fluorescence was used to assess adsorption of PAHs to lenses. Lenses were dissected from field-collected spotted gar (*Lepisosteus oculatus*) and were exposed to PAHs in crude oil water accommodated fractions in the presence or absence of UV (12 h/d) for 24 h. Absorbance and fluorescence were measured using a BioTek Synergy 2 multi-mode microplate reader at 48, 72, 96, and 120 h. Optical density of lenses significantly increased following co-exposure to PAHs and UV at 96 and 120 h, indicating an effect of photo-induced toxicity. Increased fluorescence was also observed in lenses following crude oil exposure, indicating adsorption of PAHs to tissue. These results provide a novel endpoint of crude oil photo-induced toxicity in fishes and are important for understanding the effects of oil on fishery resources and for improving future oil spill response and recovery.

02.04.16 Influence of Different Catchment Features on Stream Water Quality in an Intensive Agricultural Watershed

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Streams are nested, hierarchical structures wherein the larger scale characteristics constrain the smaller components, determining instream ecology. A watershed-scale study is necessary to effectively analyze and compare the complex interactions that influence stream water quality. Few watershed-scale studies have been conducted and those that have been conducted have focused on land use as opposed to the range of variables that may influence stream health (i.e., riparian buffer extent, soil type). The objective of this study was to assess the influence of different landscape and instream characteristics on water quality across a gradient of agricultural intensity in the Grand River watershed in Southern Ontario. Twenty-one sites across the central watershed were sampled in October 2020 and June 2021. A micro-basin polygon was generated for each site wherein land drainage at each site was mapped and analyzed for spatial heterogeneity (i.e., land use, surficial geology, riparian buffer extent) derived using a geographical information system. At each site, benthic macroinvertebrate assemblages were sampled, water chemistry samples collected and/or measured directly, and habitat quality assessed. It is hypothesized that micro-basins with a greater proportion of agricultural best management practices (i.e., greater buffer extent, cover crop presence) would contain better water quality as indicated by the water chemistry measurements and macroinvertebrate community composition. Shannon diversity index values for the October 2020 invertebrate data ranged from 1.5 to 2.7, species richness ranged from 18 to 33, and the Ephemeroptera, Plecoptera, Trichoptera (EPT) index ranged from 10 to 50. Greater diversity, richness, and percent EPT values were associated with micro-basins that had a lower gradient of agricultural land use and greater riparian buffer presence, suggesting that land use and buffer extent play a large role in determining stream health. Similarly, non-metric multidimensional scaling (NMDS) using Bray-Curtis indices of dissimilarity showed a strong correlation between greater relative area of riparian buffer and percentage of forested land use within the micro-basin to macroinvertebrate families with lower pollution tolerance values. An enhanced understanding of the landscape characteristics that most significantly influence water quality will help watershed managers provide accurate recommendations for upstream catchments to preserve stream health.

02.04.17 Prioritizing Organic Waste Chemicals and Locations of Ecological Concern in Sediment From Great Lakes Tributaries Using Multiple Lines of Evidence

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Environmental monitoring studies frequently describe contaminant occurrence without regard to their biological relevance. For this study, organic waste chemical (OWC) occurrence was examined alongside their potential biological effects, individually and as mixtures, as well as the biological pathways they may influence. Sediment was collected at 71 Great Lakes tributary sites and analyzed for 87 OWCs. Chemicals and locations of concern were prioritized by comparing sediment concentrations and estimated porewater concentrations to established whole-organism benchmarks (i.e., sediment and water quality criteria and screening values), and high-throughput toxicity screening data from the U.S. Environmental Protection Agency's ToxCast database. Sediment quality benchmarks were exceeded for 38 chemicals, water quality benchmarks (for pore water) were exceeded for 32 chemicals, and ToxCast screening values were exceeded for 39 chemicals. Collectively, at least one benchmark/screening value was exceeded for 54 of the 87 OWCs, with exceedances observed at all 71 of the monitoring sites. Chemicals with the greatest potential for biological effects, both individually and as mixture components, were bisphenol-A, 4-nonylphenol, indole, carbazole, and several polycyclic aromatic hydrocarbons (PAHs). Alkylphenol mixtures exceeded an alkylphenol toxicity quotient at seven sites, and PAH mixtures exceeded both the probable effect concentration and the sum equilibrium partitioning sediment benchmark toxicity units at eleven sites, indicating the potential for adverse effects due to these classes of chemicals. Potential adverse outcomes based on ToxCast gene targets and putative adverse outcome pathways relevant to individual chemicals and chemical mixtures included tumors, skewed sex ratios, reproductive dysfunction, hepatic steatosis, and early mortality, among others. Results provided a prioritization of chemicals with the greatest potential for adverse biological effects and an indication of sites where they are most likely to occur. The contents of this abstract neither constitute, nor necessarily reflect, USEPA policy.

02.04.18 Application of Toxicological Models to Identify Safe Mixtures

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While collecting data for the derivation of Biotic Ligand Model (BLM)-based copper criteria, a permitted discharger suddenly began failing whole effluent toxicity (WET) tests. The permitted discharger had historically been in compliance with their WET permit limits, however the sudden onset of WET toxicity corresponded with variability in the BLM input parameters. This onset of variable water chemistry sent the BLM, which is a probabilistic model, down a more conservative path. An initial review of water chemistry found that osmotic stress and elevated copper were likely contributing to toxicity, however, the cause of the sudden shift in water chemistry was not clear. Furthermore, the treatment that previously reduced copper bioavailability was no longer providing consistent water chemistry. In order to meet permit limits, further assessment indicated that additional treatment was needed in order to return water chemistry to non-toxic ranges. The selection of an appropriate treatment technology required an identification of a range of concentrations for both copper and major ions within which non-toxic conditions were expected to be supported. To determine the non-toxic target concentrations, toxicity models were applied (i.e., Salinity-Toxicity Relationship and BLM). This presentation will step through our use of various toxicity models to determine safe concentrations of complex mixtures with the goal of identifying non-toxic target concentrations for compliance.

02.04.19 Assessment of Potential Effluent Toxicity From Ash Basin Dewatering Operations

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Dewatering of ash basins, as part of facility surface impoundments compliance with the Coal Combustion Residual (CCR) Rule, often generates wastewater treatment effluents with elevated levels of total dissolved solids (TDS). These effluents are typically monitored for individual concentrations of various cations (e.g., arsenic, copper, nickel, zinc) or anions (e.g., sulfates, chlorides), but are seldom evaluated in their entirety. For facilities that have whole effluent toxicity (WET) limits in their National Pollutant Discharge Elimination System (NPDES) permits, this may lead to compliance issues, as elevated TDS may be toxic to aquatic organisms. Model organisms used for WET tests, the water flea, *Ceriodaphnia dubia*, and the fathead minnow, *Pimephales promelas* are particularly sensitive to effluents with elevated levels of TDS or specific cations and anions. These effluents may be acutely or chronically toxic, depending on effluent volume and the receiving water flow. This presentation provides an overview of the acute and chronic toxicity of various cations and anions representing TDS of ash basin dewatering effluents to standard organisms used in WET testing. We discuss the benefits of an acute and chronic toxic unit approach for understanding cumulative toxicity of this effluent to standard toxicity test organisms, emphasizing scenarios where using previously collected data is necessary. This presentation will also provide strategies for performing toxicity reduction evaluations (TRE) on ash basin effluents that have been identified as unacceptably toxic and the evaluation of potential impacts to receiving waters. Considerations will be discussed for addressing complications observed during toxicity testing performance resulting from ion imbalances in some ash basin dewatering effluents and the associated receiving waters.

02.04.20 Comparison of Susceptibility to Copper and Predation Stress Between Coastal Copepod Species

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Copper pollution is an increasing problem along the Norwegian coast due to its use as an antifouling agent in aquaculture. It is well established that high copper concentrations are toxic to aquatic organisms. However, most toxicity studies are performed in controlled laboratory settings with a single contaminant, not accounting for interactions between the pollutant and naturally co-occurring stressors like predation. Since the interactive effects can range from antagonistic to synergistic, multiple stressor studies can give a more realistic understanding of the effect of copper contamination on real populations. In the copepod *T. brevicornis*, predation risk had a potentiating effect on copper toxicity, prolonging development time to adult stage. To predict the overall effect of copper contamination on coastal ecosystems, knowledge of whether this effect differ between species is necessary. There is a large inter-specific variation in copper sensitivity among copepods, with the mainly benthic group of harpacticoids being less sensitive in acute (lethal) toxicity studies compared to pelagic calanoids. This study investigates the differences in sensitivity to copper exposure between a benthic and a pelagic copepod species, singly and combined with predation risk. We will investigate both acute (time to death) and chronic (time to development to adult stage) endpoints. Environmentally relevant concentrations of copper do often not cause acute effects in copepods, therefore studying sublethal effects provides a clearer idea of how populations are affected. Effects such as increased development time can be seen at lower, more realistic pollutant concentrations, and can influence population dynamics. Furthermore, since sensitivity to acute and lethal endpoints can be very different, comparing the two can further increase our understanding of the effects. We expect pelagic species to be the most sensitive to both endpoints, due to the low background levels of copper and a naturally more constant environment. In contrast we expect benthic species inhabiting a more fluctuating environment to be more robust. With respect to the interaction with predation, we expect similar potentiating effects in both species, since in coastal regions both species should encounter fish.

02.04.21 Effects of Multiple Stressors on Copepod Populations of Temora Longicornis with Different Copper Exposure History

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Marine ecosystems are complex systems with an abundance of organisms that are continuously exposed to numerous stressors. Copepods are a zooplankton group with an integral role in the marine food web. Interactions between multiple stressors of both natural and anthropogenic origin can have a huge impact ranging from antagonistic to synergistic effects. The increasing use of copper (Cu) as an antifoulant in the aquaculture industry is a major concern for aquatic ecosystems, given its effect on non-target species. Still, there is a lack of knowledge on the severity of its effects on the marine environment, especially on copepods. Whilst being an essential element, at high concentrations, Cu induces reactive oxygen species in organisms. Astaxanthin is a pigment with antioxidative properties that copepods take up through their diet. However, this pigment also causes a conspicuous orange color in the copepods, leaving more pigmented individuals at a potential higher risk to visual predators, such as fish. A trade-off might exist between predation risk and antioxidant capacity in copepods. Our aim is two-fold: 1) to identify whether historic Cu exposure from aquaculture activity affects the survival of copepods under the combined exposure to the anthropogenic stressor Cu and the natural stressor predation risk (kairomone), and 2) whether their sensitivity to Cu exposure is related to pigmentation. We sampled six populations of the pelagic copepod *T. longicornis* from the Boknafjord/Ryfylket region (Stavanger, Norway) along a gradient of aquaculture activity. Populations were exposed for 72 hours to, Cu (150 µg/L), predation risk, combined Cu and predation risk, and a control to test how it would affect time until death and astaxanthin pigmentation. In addition to manual observations, an automated imaging robot took pictures continuously (25 min intervals) throughout the experiment to determine the exact time of death through image analysis. Astaxanthin content will be measured from each individual after the experiment. We hypothesize that populations from sites with higher historical records of high Cu exposure have an increased tolerance to acute Cu exposure and a higher degree of pigmentation to battle Cu toxicity than populations stemming from less exposed sites.

02.04.22 Sensitivity of the Early Life Stages of the Arctic Copepod C. Glacialis to Multiple Stressors: Multi-Dimensional Interactions of Ocean Acidification, Warming and Pyrene

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The Arctic is currently experiencing an increase in temperature as well as a decrease in pH due to increased concentration of CO_2 in the atmosphere. The increase in temperature will mean that the Arctic will be more open for ship traffic and petroleum activity, which will expose the Arctic to polycyclic aromatic hydrocarbons (PAH's), such as pyrene. The toxicity of many contaminants is higher under elevated temperature, while low pH from ocean acidification (OA) may add an additional effect on top of the temperature-contaminant interaction. However, the interactive effects of these stressors on the Arctic marine species are understudied. Copepods are the most abundant group of zooplankton on the earth and are important species in the ecosystems they inhabit. *Calanus glacialis* is an Arctic species and is an important food source in the arctic food web. Early life is supposed to be the most sensitive stage to single and multiple stressors. The effects of temperature, OA and pyrene on eggs and nauplii of *C. glacialis* may have direct consequences for population structure, dynamic and trophic transfer of energy and resources in the Arctic marine ecosystems In this study we will compare the single and the combined effects of the three stressors including pyrene (0nm vs 200nM), elevated Temperatures (1 vs 5°C), and ocean acidification (pH 8.05 vs 7.55), on the eggs and nauplii of unexposed *C. glacialis* females.

02.04.24 Toxicity of Sediments from Flooded Agricultural Fields to Early Life Stage Zebrafish (Danio rerio)

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Floodplains of fluvial lakes make attractive spawning habitat for many freshwater fishes. However, when flooding takes place over agricultural fields, water quality is compromised with potential repercussions to the health of fish embryos and larvae. In the present study, we measured pesticide concentrations in water and sediments collected from flooded agricultural fields, and used a zebrafish (*Danio rerio*) assay to assess their toxicity to early life stage (ELS) fish. Samples were collected from the floodplain of Lake Saint Pierre, Quebec (a fluvial lake in the Saint-Lawerence River) in early spring of 2019. Collection occurred during the yellow perch (*Perca flavescens*) spawning season, prior to planting and application of pesticides to agricultural fields. Water and sediment samples were taken from flooded fields that had been planted with soybean (n=2) or corn (n=2) the previous spring, and from geographically matched flooded forested areas (n=4) to serve as controls. Nine herbicides and insecticides were detected in water samples at concentrations ranging from 1-380 ng/L. Of these, two neonicotinoid insecticides, thiamethoxam and clothianidin, were detected at levels exceeding toxicity thresholds for aquatic life. Pesticide concentrations were similar in water collected from agricultural and forested sites. In contrast, two insecticides (clothianidin and chlorantraniliprole) and four herbicides (mesotrione, imazethapyr, glyphosate and aminomethylphosphonic acid) were detected in agricultural sediments at levels ranging from 0.9-14 ug/kg, whereas no pesticides were detected in the corresponding forest sediments. To perform the zebrafish contact assay, sediment (35 g) from each treatment was placed in glass beakers and filled with reconstituted hard water (4 replicate beakers/sediment type). Each beaker contained a glass cylinder with nylon mesh (100 µm) suspended several inches from the bottom. Zebrafish embryos (n=40/beaker) were placed on the mesh and exposed from ≤ 2 hours post fertilization (hpf) to 120 hpf. Preliminary results suggest that survival and time to hatch were significantly decreased in fish exposed to sediments from one of soybean fields compared to its geographically matched forest control ($p < 0.05$). However, sediments from both these sites caused a depletion of oxygen levels in test beakers, complicating interpretation of the data. Analysis of the remaining samples is ongoing.

02.04.25 A Preliminary Ranking of Contaminants of Concern in Juvenile Harrison Chinook and Their Habitat in the Fraser River, British Columbia

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The Fraser River is the largest river in British Columbia and was once considered the world's most productive salmon river. This river is home to 19 Chinook populations which represent an important species not only to First Nations, recreational anglers and commercial fisheries but also to Resident killer whales. The Harrison River Chinook stock, which numerically dominate the lower Fraser River stocks, spend 30-50 days in the Fraser River estuary, where they feed and grow prior to entering the marine environment. This stock was selected as a focal stock due to its spawning location origin in the lower Fraser River, and because ocean type Chinook are believed to rely most heavily on estuarine habitat compared to "stream type" Chinook and other salmonid species. The lower Fraser River and estuary is heavily impacted by a number of anthropogenic activities including, but not limited to, forestry, mining, pulp and paper, wood preservation, chemical manufacturing, urban and agricultural runoff and wastewater (sewage) treatment. Despite a history of pollution inputs into the lower Fraser River and estuary, little is known about contaminant exposure and their associated health impacts in Chinook salmon. A preliminary ranking of over 400 contaminants of concern, including various pesticides, PCBs, PBDEs, flame retardants, personal and pharmaceutical care products, and metals in Harrison juvenile Chinook and their habitat (water and sediment) was conducted. Whole juvenile Chinook salmon composites, water (monthly, April to June) and sediment (annually) were collected from three impacted sites within the estuary and two reference sites with relatively lower to no anthropogenic impacts. A combination of exposure activity ratios, risk quotients, and toxicity quotients were applied across the different matrices. This initial ranking of contaminants will serve to identify those contaminants suspected of driving observed health effects in the real world that will be further explored in a field health effects study and in lab-based exposure experiments. Results from this research will be used to inform Chinook conservation, recovery, and management efforts, especially as they relate to Fraser River Chinook and Southern Resident killer whales.

02.04.26 Bioaccumulation of Lead in Three Fish Species of the Upper Cache River Watershed, Arkansas Upper Cache River Watershed, Arkansas

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Lead (Pb) is a ubiquitous metal that has been shown to the impact health of higher trophic levels. Pb is most prevalent in sediments, but it can also be found in aquatic environments. In freshwater systems, Pb may become bioavailable when water conditions are slightly acidic and soft. While Pb does not biomagnify in freshwater fishes, it has been shown to bioaccumulate in various tissues including the blood, liver, bones, and muscle. Streams and soils of agricultural lands have greater concentrations of Pb than non-agricultural areas. In the Cache River Watershed, approximately 70% of the 230-km-long watershed is used for agricultural purposes with over 200 river-km listed as impaired for not supporting aquatic life due to agricultural sedimentation. Several of these stream segments are also listed for Pb impairment. The goal of this project was to determine if Pb is bioaccumulating in *Gambusia affinis, Lepomis cyanellus*, and *L. macrochrius* and if the bioaccumulation is greater in subwatersheds with high agricultural intensity. Five to 10 fish of each species were collected from 12 subwatersheds of the Upper Cache River Watershed, digested following a modified EPA method 3050b, and analyzed on a graphite furnace atomic absorption spectrophotometer. Preliminary results show, when grouped by agricultural intensity, the presence of Pb in fishes is greatest in low and moderate high intensities. Of the three fish species sampled, *Lepomis cyanellus* had the greatest concentration of Pb. Based on the current Interim Reference Level (IRL) of 12µg/day, consumption of fishes from the Cache River Watershed are below the IRL set by the FDA.

02.04.27 Comparison of Water Quality Measurements of the Bayou DeView River and Tributaries in Northeastern Arkansas, USA

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Rowcrop agriculture is prevalent in Northeast Arkansas and the Cache River Watershed landuse is primarily cropland dominated by rice and soybeans. Rowcrop agriculture is the predominant landuse (80%) in Cow Lake Ditch, a subwatershed of the Cache River. Water quality monitoring for pH, conductivity, dissolved oxygen (DO), turbidity, total suspended solids (TSS), total and dissolved nitrogen and phosphorus has been collected within each of the seven sub-watersheds of Cow Lake Ditch since October 2016 and three of these locations are listed as impaired for DO, sulfates, and turbidity. Comparisons over four water years, designated by the USGS as Oct – Sept, were made within each subwatershed. Variations in precipitation amount and intensity varied over the study years. Riparian vegetation changed greatly within the Cow Lake Ditch subwatershed with the removal of the forested riparian buffer during year 2. Riparian tree removal and dredging within this subwatershed resulted in a 79% increase in mean turbidity values in year 2. Turbidity values decreased in year 3 to values similar to year 1; however, a 30% increase from year 1 was measured in year 4. Long-term water quality monitoring provides an opportunity to measure changes throughout different water years and evaluate water quality variations in each subwatershed.

02.04.28 Effects of Park Closure on Water Quality in the Buffalo National River, AR

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In 1972, the Buffalo National River (BNR) was declared the first National River in the United States, however, it has recently been listed as one of the most endangered rivers in the country. In 2018, a section of the Buffalo River's mainstem was listed as impaired for pathogens, and a tributary of the Buffalo River was listed as impaired for low dissolved oxygen and pathogens. During the 2020 coronavirus pandemic, the National Park Service (NPS) closed the park to recreational activities from April 2 to May 15, 2020. Visitor use is a potential contributing source for nutrients and bacteria to BNR, and the closure offered an opportunity to study the effects of visitor use on the river's water quality. The NPS and Arkansas State University collaborated to analyze samples collected semiweekly through July 9, 2020, several weeks after reopening, and weekly sampling through September 28, 2020. Sampling sites included upstream/downstream of three popular campsites, a spring at one of the campsites, and a tributary associated with one of the campsites. Samples were analyzed for turbidity, *Escherichia coli,* and total and dissolved nutrients. Although there were no significant differences between upstream and downstream of the campgrounds, the greatest turbidity and *E. coli* values were measured following storm events, indicating that storm runoff increases these contaminants entering the waterways. Analyses of nutrients data showed a general decrease after the park closure, with the exception of increased concentrations following storm events. The ability to analyze effects of tourism on the BNR can aid in the understanding of contaminant contribution to the nation's first National River.

02.04.29 Pilot Application of New Chemical and Toxicological Tools to Characterize Water Quality Stressors

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States and Tribes are required to assess waterbodies and identify water quality impairment causes, but stressor identification is often unsuccessful. Emerging bioeffects-based tools may support routine monitoring and stressor identification. Identifying affected molecular targets may aid determination of adverse outcome pathways and, if integrated with extensive chemistry data, the causative chemicals. This study applied *in vitro* bioassays (Attagene, Inc.) and targeted and non-targeted chemical analyses in a biologically impaired stream with poorly characterized stressors and several potential contaminant sources (e.g., upgradient agriculture, stormwater outfalls, and a downgradient wastewater treatment plant (WWTP)). Eight sites representing these sources and routine biomonitoring locations were sampled in 2019. Low- (no recent rain) and high-flow (storm runoff) conditions were sampled to assess runoff influence on sites' chemistry and ecotoxicology. Chemical data confirmed that agriculture, stormwater, and the WWTP are CEC sources and that their profiles reflect expected chemical uses and occurrences. Bioeffects-based source and site signatures were less distinct; most samples activated a handful of targets, especially the receptors for pregnane X receptor (PXR) and aryl hydrocarbon (AhR). These results parallel a recent nationwide survey that used Attagene methods. WWTP effluent activated the most targets (including estrogen and glucocorticoid receptors). Runoff had substantive effects on the chemical and bioeffects profiles throughout the system, including PXR, AhR, and retinoic acid receptor-beta (RXRβ) activation at all "in-town" in-stream and stormwater sites. RXRs and AhR are thought to affect fish embryo development; specific effect (i.e. cardiac function) analysis is underway. Because the consequences of PXR and RXRβ activation are poorly characterized in fish, the risks associated with their activation herein are difficult to predict. Given the general lack of diversity of molecular bioeffects across sites, and because PXR, RXRβ, and AhR are activated by many chemicals, we concluded that quantitative integration of chemical and bioeffects data is unlikely to identify stressors in this case. We are currently completing exploratory multivariate analysis of chemical and biological data, and integrating chemical-specific bioeffects data (from the CompTox Chemicals Dashboard) with our data to prioritize known chemicals and sources in this system.

02.05 Bioaccumulation: Linking Exposure to Effects in Aquatic Ecosystems – the Use and Application of Bioaccumulation Testing

02.05.01 Passive Dosing with Biocompatible Polyethylene Meshes to Control Exposure and to Measure Toxicity and Bioaccumulation in H. Azteca Water-Only Tests

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Chemical exposure in aqueous toxicity tests with the amphipod *Hyalella azteca* can be poorly defined when applying conventional solvent spiking methods, especially for hydrophobic organic chemicals (HOCs) that are complicated to solubilize in water and sorb to vessel materials, to food particles, and to the plastic mesh that is commonly applied as a shelter for the amphipods. We assessed the suitability of biocompatible plastic meshes as passive dosing reservoir to control freely dissolved (C_{free}) and total aqueous concentrations ($C_{w,total}$) of nine HOCs in the acute 96-hour *H. azteca* toxicity test. In addition to concentration-response testing, the developed method was applied to evaluate the contribution of food particle and dissolved organic carbon (DOC) ingestion to the HOC uptake in bioaccumulation experiments. Of three polymers tested, polyethylene showed the highest capacity for the test chemicals and was chosen as passive dosing reservoir. Mixture experiments with four polycyclic aromatic hydrocarbons demonstrated that ≤ 11 hours of incubation of pre-loaded polyethylene meshes in test water is sufficient to achieve equilibrium, and both C_{free} and $C_{\text{w,total}}$ remained constant in the subsequent 96-hour toxicity test. In contrast, $C_{\text{w,total}}$ of all chemicals were lower than intended nominal concentrations and decreased over time using conventional solvent spiking. As a consequence, solvent spiking resulted in a lower apparent toxicity of permethrin compared to passive dosing. In all experiments, $C_{\text{w,total}}$ of hydrophobic chemicals was higher than corresponding C_{free} following chemical sorption to food particles. Bioaccumulation experiments at constant C_{free} and varying dosages of food and a sediment DOC surrogate (peat moss suspension) indicated that uptake of bound chemicals by ingestion and/or filtration can contribute to total body concentrations of hydrophobic chemicals. Our results emphasize the necessity of using passive dosing in *H. azteca* water-only tests to establish constant exposure conditions of HOCs while considering both the freely dissolved and bound species in the interpretation of toxicity and extrapolation to environmental exposure. The fast release kinetics of chemicals, the beneficial effects on amphipod fitness, and the ability to adjust defined C_{free} and $C_{\text{w,total}}$ suggest using plastic meshes to adjust exposure conditions comparable to sediment toxicity tests.

02.05.02 Pesticide Bioavailability and Bioaccumulation in Juvenile Chinook Salmon, Prey Items and Sediments of the Sacramento River Watershed

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The Sacramento River watershed provides an important rearing habitat for juvenile Chinook salmon prior to outmigration. Previous studies have demonstrated advantages for fish rearing in the Yolo Bypass, a seasonally inundated floodplain, as compared to the adjacent mainstem Sacramento River. In addition, previous gut content analysis has suggested different sources of basal energy between the two systems, with greater prevalence of benthic prey items in the floodplain as compared to more pelagic feeding in the mainstem. Due to the hydrophobic nature of anthropogenic contaminants including legacy and current-use pesticides, benthic prey items may bioaccumulate greater amounts of contaminants as compared to pelagic prey, posing a greater risk to consumers, such as juvenile Chinook salmon. To elucidate the potential for dietary pesticide exposure in juvenile salmon rearing in the Yolo Bypass and Sacramento River, sediments, benthic and pelagic dietary items and juvenile Chinook were collected in 2019 and 2020 and analysed for a suite of pesticides. To assess bioavailability of selected pesticides in sediments, single-point Tenax extractions (SPTEs) were used as well as accelerated solvent extraction (ASE) to measure total pesticide concentrations. The most common analytes detected were DDT, DDE, and the pyrethroid insecticide bifenthrin. The number of bioavailable pesticides detected was significantly lower in 2020 as compared to 2019, which may have been related to differences in hydrological conditions between years. A significantly greater number of contaminants were detected in zooplankton as compared to macroinvertebrates across both areas, driven by increased detections of phenylpyrazoles and pyrethroids in zooplankton. Zooplankton collected from the Yolo Bypass exhibited a significantly greater number of chlorpyrifos detections as compared to the Sacramento River. Analysis of juvenile Chinook salmon is currently ongoing and will be included within this presentation, with preliminary data suggesting the presence of organochlorines and pyrethroids in juvenile Chinook. The findings of the current study suggest that dietary preferences play an important role in determining the pesticide exposure of rearing juvenile Chinook. The results of the present work will elucidate the benefits for juvenile fish rearing in floodplain habitats, and aid in mitigating the potential effect of contaminants on threatened juvenile salmon populations.

02.05.03 Potential for Trophic Transfer of p,p'-DDT and Metabolites to Juvenile Chinook Salmon (Oncorhynchus tshawytscha) From Consumption of Chironomus dilutus

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Many populations of Chinook salmon (*Oncorhynchus tshawytscha*) in the western United States have experienced large declines in spawning numbers in the last decade. In the Central Valley, CA, both legacy and current-use pesticides have been detected in known Chinook dietary items, including the midge, *Chironomus dilutus*. Both larval and adult midges have been shown to be the most common dietary items for rearing juvenile Chinook in the Sacramento-San Joaquin Delta, however the capacity for contaminant uptake and biotransformation among midge life stages is poorly understood. One of the most commonly detected compounds in Chinook salmon rearing habitat is p,p'-DDT, its isomer, o,p'-DDT, and its biotransformation products, p,p'-DDE and p,p'-DDD. This study aims to observe bioaccumulation and biotransformation of p,p'-DDT across all life stages of midges, from larvae to flying adults, and assess the potential for trophic transfer to Chinook. First instar midges will be exposed to p,p'-DDT-spiked sediment and exposure will continue throughout their life cycle at a sublethal concentration. Midges will be removed at each life stage (2^{nd},3^{rd},4^{th}, pupae, and adult) to measure bioaccumulation and biotransformation products. Next, the life stage with the highest concentration will be fed to juvenile Chinook salmon for 7 days to simulate dietary uptake during rearing. Bioaccumulation of p,p'-DDT and biotransformation products will be assessed in juvenile Chinook, elucidating the potential for trophic transfer of contaminants and risk to rearing juvenile salmon. These studies are currently ongoing in our lab, and it is anticipated that midge and salmon bioaccumulation and biotransformation data will be presented. Through this study, we aim to compare and contrast the different rates of bioaccumulation and biotransformation though each midge life stage and the resulting trophic transfer from midges to juvenile salmon. We expect the highest uptake and biotransformation to occur in 4^{th} instar individuals, followed by decreases in update and biotransformation as midges pupate. Additionally, we expect that these compounds will be transferred to juvenile salmon through this feeding exposure. These findings will elucidate the potential risk for juvenile Chinook salmon feeding on midges throughout their life cycle.

02.05.04 Evaluating 40 Years of Sediment Bioaccumulation Testing with Lumbriculus variegatus

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To evaluate the aquatic risk of a contaminant, an understanding of both exposure and effects are needed. Traditionally, chemical concentrations in a media (exposure) are compared to toxicity bioassays with the media (effects) to understand risk. These evaluations, however, take a considerable amount of interpretation as each determination is conducted separately. The use of bioaccumulation assays in aquatic assessments are an invaluable tool in linking the two methods as accumulation of chemicals in biota provided a direct link from exposure to effects. This is especially true for the assessment of sediments (which many have speculated are the most important route of exposure for benthic organisms), as bioaccumulation assays take into consideration both ingestion and dermal adsorption exposure routes. Although sediment bioaccumulation assays started in the mid-1980s, it wasn't until 1994 (with the second edition in

2020) that the U.S. Environmental Protection Agency chose *Lumbriculus variegatus* as the model freshwater organism for assessing bioaccumulation in sediments. The current study evaluated published papers from 1980 to 2020 pertaining to sediment bioaccumulation and *Lumbriculus variegatus*. In this review, we explored the differences between field and laboratory spiked sediment bioassays, the trends for bioaccumulation testing for individual and chemical classes, the types of bioaccumulation assessments and kinetic modelling utilized, as well as more in-depth assessments of individual chemicals (i.e. benzo(a)pyrene). Overall, the goal of this review was to both identify the current data gaps and limitations with current sediment bioaccumulation testing, but also understand the advances of sediment bioaccumulation since its initial use in the early 1980s.

02.05.05 Assessment of In Vitro Intrinsic Clearance in Various Regulatory Fish Species and the Prediction of Bioaccumulation

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Incorporating metabolism in fish is critical to improving bioaccumulation estimates, particularly those produced utilizing many of the existing computational models. The *in vivo* OECD 305 fish bioconcentration factor (BCF) guideline does account for metabolism but it is costly, labor and resource intensive and requires large numbers of fish. An *in vitro* testing strategy is needed to close the gap between *in silico* (QSAR) and *in vivo* (OECD TG 305) approaches. Recent mandates in the European Union and other geographies, to reduce the number of vertebrate organisms used in regulatory testing, have spurred the development of an *in vitro* method to predict fish bioaccumulation (OECD Test Guideline 319B, Determination of *in vitro* intrinsic clearance using rainbow trout liver S9 sub-cellular fraction (RT-S9)). However, at present, there is still a lack of data regarding this methodology and the ability to predict BCF in regulatory fish species other than rainbow trout, a representative of cold water fish species and used in the OECD TG 319A and 319B Ring Trial. In this study, we determined *in vitro* metabolism rates of seven chemicals across multiple classes of chemistry and modes of action in rainbow trout, bluegill, carp, largemouth bass and fathead minnow liver S9 sub-cellular fractions. While this method is limited to liver metabolism and there are multiple pathways in which a fish could metabolize exogenous test substances, our results indicate that the *in vitro* intrinsic clearance method using liver S9 fractions from various species presents a promising alternative method to the *in vivo* fish bioaccumulation study. The OECD TG 319A and 319B provide the basis for a robust method that can be applied and modified to other fish species as well as other organisms. Further *in vitro* to *in vivo* modeling will be required to predict the bioconcentration factor (BCF) according to each species. At minimum, these data will demonstrate biotransformation and clearance rates of chemicals in other species and data utilized in platforms such as the Bioaccumulation Assessment Tool (BAT).

02.06 Canada's Oil Sands and Dilbit

02.06.01 Using Métis Knowledge and Quantitative Inquiry to Investigate Contaminant Exposure in Freshwater Mussels in the Oil Sands (Alberta, Canada)

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Oral history shared by the McMurray Métis and other Indigenous Knowledge holders in the oil sands region of Alberta indicate that the gathering and eating of freshwater mussels (referred to locally as clams; Unionidae), have historically been part of traditional cultural practices and are an important indicator of the health of the water. The McMurray Métis are known as the River People; the river is part of who they are just as they are part of the river. Ceremony and their traditional practices are used to maintain good relationships to engage with Mother Earth and all her creatures in respectful and reciprocal ways. However, McMurray Métis Knowledge holders have noticed that clams are not nearly as abundant and widespread as they were to 20-40 years ago. Led by the McMurray Métis, in partnership with Alberta Environment and Parks and Environment and Climate Change Canada, we co-developed a framework to guide how Indigenous Knowledge and Western science would be ethically and practically situated in the research and then co-designed an investigation into the health of freshwater mussels in the oil sands region of Alberta. As a first step, the McMurray Métis decided to focus on using Indigenous Knowledge to find clams on the Athabasca and Clearwater rivers and to quantify potential contaminant levels (metals) in freshwater mussel tissue, sediment and surface water. Western science was used to support this line of questioning through place-based interviews and quantitative inquiry. Through our ethic of 'Learning Together' we touch on cultural impacts to McMurray Métis as a result of the decline of freshwater mussels in the region and show that the concentrations of numerous metals including aluminum, cobalt, nickel, and lead, were higher in freshwater mussel tissue, sediment and water collected from sites located on the bitumen deposits on the Clearwater River compared to samples collected off the bitumen deposit. While, fewer samples have been collected to date from the Athabasca River, metal levels in sediment and tissue were generally higher than those collected from the Clearwater River. Results from this research can be used to help better understand contaminant distribution and effects on aquatic health in the Athabasca and Clearwater rivers. As well, this work shows that when Indigenous Knowledge is prioritized in 'a good way', we have a more holistic understanding of the environment while continuing to work towards decolonizing research.

02.06.02 Evaluating Lower Athabasca River Sediment Metal Concentrations from Alberta Oil Sands Monitoring Programs Using Pre-Development Baselines

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Since 1997, sediment metal concentrations have been monitored in the Alberta Oil Sands Region (AOSR) of the Lower Athabasca River by the Regional Aquatics Monitoring Program (RAMP; 1997-2002), Joint Oil Sands Monitoring Program (JOSM; 2012-2014), and Oil Sands Monitoring Program (OSM; 2015-present). However, it has remained difficult to differentiate industrial from natural sources and quantify the extent of pollution due to inadequate knowledge of pre-development reference conditions. Here, baselines were constructed using pre-development (i.e., pre-1967) sediment concentrations of USEPA priority pollutants (Be, Cr, Cu, Ni, Pb) and V, an element elevated in bitumen and associated waste materials, normalized to Al concentration in cores from floodplain and upland lakes within the AOSR to characterize the natural range of variability. The Lower Athabasca River sediment metal monitoring data were examined in the context of the pre-development baselines. Most metals are below the threshold for minimal enrichment (< 1.5x baseline) except for chromium (up to 4.8x) in some RAMP samples. The pre-development baselines for sediment metal concentrations will be of particular importance as the oil sands industry potentially shifts from no-release policy to treatment and release of oil sands process waters directly to the Lower Athabasca River.

02.06.03 Using Engineered Floating Wetlands to Bioremediate Oil Spills in Freshwater Shoreline Environments at the IISD - Experimental Lakes Area, Ontario, Canada

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Canada has the third largest crude oil reserve in the world and produces more oil than consumed locally, resulting in the transport and export of oil across Canada. When oil is accidentally released into freshwater environments it is conventionally treated with mechanical recovery methods. These methods generally only recover 20-40% of spilled oil and can be damaging to sensitive shoreline habitats. Biological recovery methods may be a less invasive alternative for oil spill recovery in sensitive habitats and are often more cost effective. Research at the International Institute for Sustainable Development Experimental Lakes Area in Northwestern Ontario, Canada, is assessing the use of engineered floating wetlands (EFWs) as a non-invasive, secondary recovery method to naturally stimulate microbial biodegradation of oil in experimental shoreline enclosures in a boreal lake. EFWs are vegetated floating platforms that allow plants to grow a dense root surface area and release plant exudates that can support a diverse microbial community within the rhizosphere. In 2019, EFWs were assessed for oil spill recovery after a controlled addition of diluted bitumen in wetland and rock cobble shoreline enclosures through monitoring changes to the total polycyclic aromatic compounds (PACs) and the rhizosphere microbial biofilm community using rRNA gene amplicon sequencing. Preliminary results revealed that total PACs peaked on day 20 of exposure at 12,440.83 ng/L and declined to 377.78 ng/L on day 87 of exposure in the wetland shoreline experimental enclosure. Preliminary analyses of the rhizosphere microbial community revealed that the EFWs supported a diverse and variable microbial community including some taxa previously identified to have hydrocarbon degrading capability. The prokaryotic community was dominated by *Proteobacteria* followed by *Bacteroidetes* in all sample periods and the eukaryotic community was dominated by *Annelida* in all sample periods and sites, and included variable abundance of *Bryozoa*, *Ciliophora*, *Phragmoplastophyta*, and *Rotifera*, among others between sites and sample periods. Further detailed analyses on microbial relative abundance and species selection upon oil exposure will be presented. Results from this research will provide much needed guidance to spill responders and environmental managers for including EFWs as tools for effectively treating sensitive shorelines affected by oil spills.

02.06.04 A Comparison of the Aquatic Toxicity and Degradation of a Diluted Bitumen to a Conventional Medium Crude Oil in Freshwater During a Two-Month Natural Weathering Test

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Bitumen is a highly degraded, viscous, unconventionally-produced form of petroleum that is blended with lighter oils to yield a less viscous diluted bitumen for pipeline transportation. Work has been underway over the last few years to develop an understanding of how the fate and behaviour of diluted bitumen products after spills in aquatic environments may differ from those of more familiar conventional crudes. However ecologic risk assessments of even the conventional crude oils are complicated by the changing compositions of organic compounds in water over time due to the changing composition of the oil (floating or submerged) as it weathers. Oil weathering includes processes such as evaporation, dissolution, dispersion, biodegradation, and photo-oxidation which cause changes to the concentrations of polycyclic aromatic hydrocarbons (PAHs) and toxic congeners present in oils. The Natural Resources Canada (NRCan) research facility at CanmetENERGY Devon has been performing experiments in a pilot-scale spill tank using 1,200 L of river water to examine the evolution of various oil/water mixtures at varying temperatures and with wave-action. Separate two-month experiments were conducted with a conventional medium crude oil and diluted bitumen in river water with pre-mixed 2,000 ppm river flood-plain sediment. Test conditions included a pattern of wave-on and wave-off actions at an average water temperature of 24°C. Water samples were collected from the tank on days 1, 6, 14, 21, 28, 35, 42, 49 and 56 to measure changes in the concentrations of a suite of parent and alkylated PAHs, volatile organic hydrocarbons and oxidized organic compounds. Newly fertilized fathead minnow (*Pimephales promelas*) embryos were exposed for 6 days to serial dilutions of the water-dissolved fractions of either the diluted bitumen or conventional crude oil at each time point. Morphological endpoints included malformations, heart rate, hatching time, condition factor and mortality. Ethoxyresorufin-O-deethylase (EROD) analyses were also conducted *in vivo* to quantify CYP1 enzyme activity as a response to the bioaccumulation of petroleum constituents.

02.06.05 In Vitro Mutagenicity of Alberta Oil Sands Tailings Pond Bitumen in FE1-Muta™Mouse Lung Epithelial Cells

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Oil sands mining activities in the Alberta oil sands region (AOSR) have polluted the surrounding environment with polycyclic aromatic compounds (PACs). However, the effects of the majority of AOSR-related PACs and mixtures have not been evaluated for genotoxicity in wildlife. One route of PAC exposure to AOSR wildlife are tailing ponds, which contain by-products of bitumen extraction processes. Previously, AOSR PACs have been shown to increase mutation frequency in wildlife. Here, we investigate the genotoxic effects of PAC mixtures derived from bitumen originating from tailing ponds in an *in vitro* mammalian mutagenicity assay – FE1 MutaMouse lung epithelial cell line (FE1). FE1 cells will be exposed to extracts prepared from AOSR bitumen, and the frequency and types of mutations will be determined in the *lacZ* transgene. Dose-response modeling of *lacZ* mutation frequency will be used to determine if mutations are induced at environmentally relevant concentrations. The types of *lacZ* mutations induced by the bitumen extract (i.e. its mutation spectrum) will be determined by high throughput DNA sequencing. We will also conduct RNA sequencing to identify other induced toxicity pathways and compare their dose-response to the mutagenic endpoints. Thus, we will characterize the *in vitro* genotoxic potency, including the induced mutation spectrum, relative to other toxicological pathways affected by bitumen derived PAC mixtures. Overall, this study will increase current knowledge and understanding of PAC mixture toxicity in the AOSR.

02.06.06 An Investigation of Chronic Trace Element Toxicity of Lake Miwasin Water and Sediment to Daphnia magna

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Lake Miwasin is a constructed pilot scale pit lake containing treated oil-sands tailings from the extraction of hydrocarbons from Alberta's bituminous sands. It is hoped that such lakes will ameliorate the toxicity of tailings, facilitating both the release of associated waters back into the environment, and the incorporation of pit lakes back into the natural 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 metal components of tailings and oil-sands process-affected waters. The immediate objective was to determine the initial (i.e. year 1 and 2 following lake construction) toxicity of Lake Miwasin water and sediment to a model freshwater invertebrate species, *Daphnia magna*. Acute 48 h toxicity tests were conducted with mortality as endpoint, and chronic 21 d toxicity tests were performed using reproduction, body mass, time for first brood, total number of broods, and number of neonates at last brood as endpoints. Acute toxicity test results showed no mortality observed over 48 h. However, in the chronic tests, final body masses of *D. magna* were significantly larger in Lake Miwasin water only treatments, compared to exposures to both water and sediment. Daphnid reproduction was also affected, with reduced total neonate production observed in both water and water/sediment groups relative to artificial laboratory water controls. Trace metal body burdens were also analyzed to learn more about the relationship between exposure and uptake by the organisms. Results showed exposure to Lake Miwasin water and water/sediment increased trace metal (i.e., Al, V, Co, Ce) burdens in daphnids. Lake Miwasin is still at an early stage and will change over time. Developing a better understanding of the toxicity associated with trace elements in Lake Miwasin will be important for assessing the safety of future pit lakes and water releases.

02.06.07 Integrating Interspecies Correlation Estimation Models to the Chemical Aquatic Fate and Effects Database (CAFE), a Tool that Supports Assessments of Chemical Spills

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The Chemical Aquatic Fate and Effects (CAFE) database is a tool that facilitates assessments of accidental chemical and oil releases into aquatic environments. CAFE contains aquatic toxicity data summarized in the form of species sensitivity distributions (SSDs) with associated 5th percentile hazard concentrations (HCs). Since its initial release in 2015, CAFE has been used in hundreds of chemical and oil spills. However, for many chemicals and oils, gaps in species diversity and toxicity data limited the development of SSDs, which showed most prominently in emerging oils such as diluted bitumen and biodiesel. These emerging oils (~20 oils) will be included in the next version of Web CAFE in early 2022. In CAFE's upcoming 2022 web version, some of these data gaps were also addressed with Interspecies Correlation Estimation (ICE) models. The incorporation of ICE predicted values into CAFE allowed the development of >800 new SSDs and increased diversity in SSDs by an average of 34 species. Multiple analyses showed that SSDs supplemented with ICE-predicted values generally produced HC5 estimates that were within a 3-fold difference of estimates from measured SSDs (58%−82% of comparisons), but that were often more conservative (63%−76% of comparisons) and had lower uncertainty (90% of comparisons). ICE SSDs did not substantially underpredict toxicity (< 10% of comparisons) when compared to estimates from measured SSD. With the addition of emerging oils and ICE models into CAFE's upcoming 2022 web version, more SSDs are generated, increasing CAFE's capacity to respond to chemical and oil spills.

02.06.08 Development of Criteria for Regional Environmental Effects Monitoring: Critical Effects Sizes of Fish Performance Parameters Athabasca River Tributaries

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Landscape development in the lower Athabasca River watershed. Alberta, Canada, due to mining has increased over the last 20 years and are encroaching on in-stream reference locations. This has resulted in the need for the development of Regional Environmental Effects Monitoring. Recent studies indicate that, despite the terrestrial landscape being altered, the magnitude of the response in the aquatic environment adjacent to those activities is similar to historic data. The objectives of this study were to define the normal range of fish performance indicators (within the Steepbank reference sites), and evaluate the change demonstrated in fish within the Steepbank relative to regional reference sites. Starting in 2010-2014, slimy sculpin were collected from six additional sites to assess the natural variability of fish performance indicators over time within and among the existing and the potential regional reference sites. At the time, the criteria for defining a potential reference were based on the best available decisions at the time of the original assessment and limited based on proximity to the known bitumen deposits, active mines, and the presence of the sentinel species of interest. This study attempts to identify explanatory variables which are driving variability in fish performance indicators regionally, the current analysis also includes the assessment of potential regional explanatory variables, such as geological attributes which ultimately determines the surface water chemistry. When all sites (2010-2019) were included in evaluating the relationships of fish performance indices, slimy sculpin demonstrated strong positive relationships between body weight and length, gonad weight and body weight and liver weight and body weight. However, fish age was less predictive of fish length and length was less predictive of liver weight. Analysis from this study demonstrated that variability in fish performance indices among years consistent with previous monitoring data and some regional sites (Firebag and High Hills) were similar to existing Steepbank River in-stream reference sites. Fish from the Horse River were typically different from the other sites evaluated demonstrating the range of variability in health endpoints for slimy sculpin and drivers of these site differences should be identified to help understand the documented.

02.06.09 Trophodynamic of Halogenated Polycyclic Aromatic Hydrocarbons in Biological Samples from the Athabasca Oil Sands Region

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Polycyclic aromatic compounds (PACs) represent a group of aromatic organic contaminants of both petrogenic and pyrogenic origins. They are released into the environment from anthropogenic and natural sources as complex mixtures, encompassing thousands of different aromatic, alkyl-aromatic and heterocyclic hydrocarbons containing N, S, or O- atoms. A preliminary study in our group has shown that halogenated polycyclic aromatic hydrocarbons (HPAHs) can also be detected in environmental samples from the Athabasca Oil Sands Region (AOSR). The current study aimed to evaluate the relationship between hepatic HPAHs in liver tissue from biota in a semi-aquatic food web. The food web comprised of snails, three species of fish, and North American river otter. Trophic levels were determined based on stable isotopes of nitrogen and carbon. HPAHs were identified by gas chromatography coupled with high-resolution time-of-flight mass spectrometry (GC-HRTOF-MS). We detected dibromofluorene (Br2-Fle), bromoacenaphthalene (Br-Ana), bromophenanthrene / bromoanthracene (Br-Phe/Ant), dichlorophenanthrene / dichloroanthracene (Cl2-Phe/Ant), and chlorophenanthrene / chloroanthracene (Cl-Phe/Ant). Cl2-Phe/Ant was measured in 44% of the samples, followed by Br-Phe/Ant (17%), Cl-Phe/Ant (14%), and Br-Ana (12%). Br2-Fle was not measured in any of the samples. Preliminary results suggest that inter-site differences were prominent for Cl2-Phe/Ant with biota collected in the oil producing regions (including steam-assisted gravity drainage and surface mineable areas) having the highest levels. While inter-species differences were negligible, our results suggest that biota at the highest trophic levels had on average the highest levels of these compounds. Future work should determine the ecotoxicological significance of exposure to Cl2-Phe/Ant in higher order vertebrates.

02.06.10 Secondary Separation and Toxicity Assessment of Bioactive Fractions from Groundwater with Influence from Natural Bitumen or OSPW Sources

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Groundwater in the oil sands region near Fort McMurray, Alberta, Canada has been identified as containing chemicals originating from sources of natural bitumen and oil sands process-affected water (OSPW). The risks to aquatic environments posed by the seepage of OSPW-derived chemicals beyond containment systems must be considered against the natural bitumen background present in the Athabasca watershed. Recent research identified fractions of dissolved bitumen-derived organics with the least polar and the most polar constituents as bioactive in acute bioassays with multiple species. Secondary fractionation using HPLC generated multiple sub-fractions, which will be used to identify the primary toxic compounds/classes within these bitumen-influenced waters, and to determine if the drivers of toxicity are different between natural and OSPW sources. Acute bioassays with *Hyalella azteca* and *Vibrio fischeri* (Microtox®Assay), species previously identified as being responsive to bitumen-derived organic toxicity, are being utilized to assess the toxicity of the isolated secondary fractions.

02.06.11 Transcriptomics Analysis of American Lobster (Homarus americanus) Larvae After Conventional Heavy Crude Oil and Polycyclic Aromatic Hydrocarbon Exposures

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American lobster (*Homarus americanus*) is the most valuable Canadian fishery, producing $1.3 B in 2016. Due to their planktonic stage, lobster larvae are vulnerable to oil spills. While it is well-known that water-accommodated fractions (WAF) of oil induce developmental toxicity through cardiac defects in fish larvae, it is not clear whether similar impacts would be observed in lobster larvae. This study aims to improve our knowledge of the molecular response of the American lobster larvae to a conventional heavy crude oil exposure. Stage I lobster larvae were exposed for 24hrs to four doses of WAF (10%, 19%, 37% and 72%) a positive control (1-methylnapthalene, at 0.3 mg/L corresponding to the estimated concentration of EC20) or a negative control (0.22 µm filtered seawater). Due to the lack of effects reported on survival, molting and respiration after WAF exposures, only the highest dose of WAF (72% WAF) was considered for transcriptomics analysis. RNA extraction of whole larvae exposed to 72% WAF (n=7), methylnaphthalene (n=6) and filtered seawater (n=6) was performed using Trizol. The transcriptomes were sequenced using Novaseq Illumina and were analyzed using EcoOmicsAnalyst (www.ecoomicsanalyst.ca) supporting the ultra-fast alignment tool for species without reference genome Seq2fun (www.seq2fun.ca). After cleaning and filtering, reads were mapped against an arthropod database. Downstream analysis including differential expression analysis and functional pathway analysis were performed using NetworkAnalyst (www.networkanalyst.ca). Preliminary results suggest that the polycyclic aromatic hydrocarbon, 1-methylnapthalene, induced more changes in the lobster transcriptome than the 72% WAF. A differential expression analysis identified 81 and 4 differentially expressed genes (DEGs) in larvae exposed to 1-methylnapthalene and 72% WAF respectively. The investigation of the effect by both treatments on the biological functions is currently ongoing. This work will contribute knowledge to understand potential impacts of oil spills on American lobster populations and to facilitate futures studies on transcriptomics in lobster larva.

02.06.12 Optimization of Plant Species Composition and Nutrient Additions to Enhance Engineered Floating Wetland Remediation of Crude Oil Spills in Freshwater Environments

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Conventional methods of cleaning up oil spills in freshwater environments are often highly invasive and can exacerbate the negative effects of the spill. One less invasive alternative is the application of engineered floating wetlands (EFWs) – vegetated floating platforms which stimulate microbial degradation of pollutants in their root zones. To support the use of EFWs in oil spill cleanup procedures, research conducted at the International Institute for Sustainable Development Experimental Lakes Area, Ontario, Canada, investigated the optimization of EFWs for removal of polycyclic aromatic compounds (PACs) from freshwater environments. Two components of EFW design were investigated: 1) the composition of three vegetation species (*Typha* sp., *Carex atherodes*, and *C. lasiocarpa*) planted on the EFWs; and 2) the carbon to nitrogen to phosphorus (C:N:P) ratio from slow-release fertilizer added in tandem with EFW application. To characterize the effects of these design parameters on oil spill remediation, twenty-six (26) 1600 L mesocosm tanks filled with lake water were exposed to equal-volume model spills of water accommodated fraction produced by weathering conventional heavy crude oil. The mesocosms were then treated with EFWs populated with varying plant species compositions or augmented with varying amounts of nutrient fertilizer. Over the duration of the twelve-week exposure, the water was monitored for PACs and nutrient chemistry, and the EFW roots were assessed for changes in the composition of the microbial community and microbial biofilm activity. The results of these analyses will be used to identify the optimal EFW treatment design to enhance the activity of oil-degrading microbes and maximize PAC removal rates to provide guidance on less invasive remediation technologies. Preliminary results on the effects different EFW designs on PAC chemistry, water nutrient chemistry, and root zone biofilm activity will be presented.

02.06.13 Characterizing the Effects of Chronic Conventional Heavy Crude Oil Exposure on the Growth, Development, and Behaviour of Larval Wood Frog (Lithobates sylvaticus)

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Much like pipelines, amphibians are distributed throughout North America spanning a variety of ecosystems. Despite an identified need for additional information, few studies have examined the effects of oil spills on the early life stages amphibians. Beginning in 2019, two parallel oil spill studies have been conducted at the IISD-Experimental Lakes Area in northwestern Ontario, Canada. The Freshwater Oil Spill Remediation Study (FOReSt) used large in-lake enclosures to determine the effectiveness of non-invasive oil spill remediation techniques in different shoreline habitats while the Floating Wetland Treatments to Enhance Remediation (FloWTER) study examined the efficacy of symbiotic plant-microbe relationships, including cattail (*Typha* sp.), coarse sedge (*Carex* sp.) and fine sedge (*Carex* sp.), for degrading oil *in situ*. We characterized the effects of chronic exposure to conventional heavy crude oil residues on wood frogs (*Lithobates sylvaticus)* in the context of both the FOReSt and FLoWTER projects. Developmental and morphometric measurements were combined with the assessment of oxidative stress markers as well as proteomic techniques to determine the effects of conventional heavy crude (CHV) exposure on tadpoles in the FOReSt enclosures. For the FloWTER project, behavioural assays were used in addition to the previously mentioned endpoints to examine sociality, activity, and predator avoidance behaviours of tadpoles. Results from this work will be presented and discussed. This research will characterize toxicological effects of conventional heavy crude on wood frogs and that can be used to inform risk assessment related to the in-land transportation of crude oil and the impacts of oil spills on amphibians.

02.06.14 Behavioural and Biometric Effects of Conventional Heavy Crude Oil on Wild Fathead Minnow Larvae from IISD-Experimental Lakes Area, Northwestern Ontario

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Global reserves of Conventional Heavy Crude Oil (CHV) are greatest in Venezuela and Canada, where pipelines are used to transport product between field and refinery, as well as to export and for consumption. Although pipelines are statistically the safest method of transportation for petroleum products, spills can occur and present a potential threat to freshwater resources. Currently remediation of freshwater oil spills includes invasive methods which may cause increased impact to sensitive, biologically productive shoreline habitats. These habitats are home to many vulnerable species including the eggs and early life stages of many freshwater fish. The Freshwater Oil Remediation Study (FOReSt) at IISD-Experimental Lakes Area is assessing the effectiveness and potential impacts of two alternative secondary remedial methods to be used in conjunction with shoreline washing and sorbent collection: engineered floating wetlands (EFWs) and nutrient enhanced monitored natural recovery (eMNR). These methods were applied to 4 large (>25,000 L) in-lake enclosures encompassing 5 m of wetland shoreline treated with controlled oil releases of 1,300 g CHV per enclosure. An additional 3 enclosures represented unoiled reference environments which also underwent shoreline washing. Wild fathead minnow (*Pimephales promelas*) eggs were collected from a nearby pristine lake and exposed in lab to water from the enclosures for 7 days post fertilization. Exposures were conducted at three timepoints during, and following, the primary and secondary remediation. Survivorship and developmental endpoints were monitored during exposure and malformations were assessed at the end of the 7d exposure. A subset of larvae were monitored for behavioural responses to light/dark stimuli and a separate subset underwent respirometry trials to determine metabolic rate. Results from this study will provide insight into the effective differences in CHV exposure and secondary non-invasive remediation methods on wild juvenile fish that may be affected by spill scenarios. Information from FOReSt will provide spill responders and environmental managers with tools to assess the applicability of less invasive oil spill clean-up methods.

02.06.15 Morphology and Oxidative Stress Markers in Lake Trout Eggs from a Lake Used to Conduct Model Oil Spills

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In Canada, and across the globe, oil production as a source of energy remains an important means of economic development, with oil and natural gas accounting for 57.5% of global primary energy consumption. While transportation safety records have steadily increased, some spills do occur. In the summers of 2018 and 2019, controlled and contained spills of diluted bitumen (dilbit) were introduced into enclosures installed in Lake 260 at the IISD-Experimental Lakes Area (IISD-ELA) in Northwestern Ontario. These additions were performed to examine the fate and behavior of oil in freshwater systems and to compare the efficacy of selected cleanup techniques as part of the Boreal-lake Oil Release Experiment by Additions to Limnocorals (BOREAL) and Freshwater Oil-Spill Remediation Study (FOReSt) studies, respectively. These projects included rigorous containment and contingency measures. The efficacy of those measures and the potential for impacts to early development of lake trout arising from exposure to residual oil was examined by comparing morphology and oxidative stress in early life stages of lake trout obtained from the lake where oil studies were conducted and unimpacted reference lakes. Lake trout are a species known to be sensitive to oil constituents, most notably polycyclic aromatic compounds (PACs). Eggs and milt were collected from fish in the four study lakes ($n = 3$-7). After transport, dry fertilizations were performed and the fish were reared in Heath trays until swim-up. A subsample of fry from each lake ($n = 6$-30 per maternal clutch) were evaluated for spinal, craniofacial, edema and finfold deformities, with no statistical difference in deformity rates being observed among lakes. Fertilization success, egg mortality and hatching success were also similar among lakes ($p > 0.05$). While no phenotypic changes were observed in fish from the lake where oil studies were conducted, we also evaluated genetic and biochemical markers of PAC exposure. Cytochrome p4501A (CYP1A), a common biomarker of PAC exposure in fish, and the ratio of oxidized and reduced glutathione, an indicator of oxidative stress, were measured in whole swim-up fry from each of the study lakes. Results from this study will be used to evaluate the efficacy of containment and contingency measures and the trajectory of recovery in oil spill impacted freshwaters.

02.06.16 The Chemistry of Oiled Water After Burning Off Introduced Slicks: The COWBOIS Study

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In-situ burning (ISB) is the controlled combustion of an oil slick to remove large quantities of spilled oil from the aquatic environment. Prior to employing ISB as a remediation technique, an oil slick must often be corralled by physical or chemical means to achieve a slick thickness of >1 mm. While ISB is acknowledged as an effective means to remove oil mass, less is known about the potential for ISB to mobilize polycyclic aromatic compounds (PACs) into the aquatic environment; PACs are the primary contaminants of concern in crude oil due to their environmental persistence and toxicity. There are conflicting reports about whether ISB removes PACs, or whether the burning facilitates mobilization of PACs into the water column. To address this question, a series of small-scale burns were conducted across a gradient of slick thicknesses (i.e. 0-7 mm). Concentrations of PACs in the underlying water were determined after the burns and compared to reference conditions using the same thicknesses of oil that were not ignited. ISB enhanced mobilization of PACs, specifically PACs of pyrogenic origin, into the water column and the mobilization was enhanced with increasing slick thickness. The potential for PAC mobilization requires particular consideration in scenarios where ISB may be the only viable oil spill remediation option (e.g. wetlands, marshes, or where oil is entrained). Understanding the ramifications of ISB can help spill responders and decision makers better prepare for oil spills. Future work should focus on the persistence of mobilized PACS, ecological effects, and the trajectory of environmental degradation under various spill scenarios.

02.06.18 Comparative Toxicity of Oil Spill Herding Agents to Seven Aquatic Species

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Herding agents are surfactant mixtures used to coalesce spilled oil and increase slick thickness to facilitate mechanical recovery or in situ burning. Only two herders are listed on the United States' National Oil and Hazardous Substances Pollution Contingency Plan (NCP) product schedule; the surface collecting agents Siltech OP-40 and ThickSlick 6535. Toxicity data for spill response agents is frequently available for only two estuarine species, mysid shrimp (*Americamysis bahia*) and inland silversides (*Menidia beryllina*) and is particularly limited for herding agents. The toxicity of oil spill response agents can vary over several orders of magnitude across product type and species, even within specific categories of spill response agents. In the current study, seven aquatic species were tested with both Siltech and ThickSlick to evaluate acute herder toxicity and relative species sensitivity. The toxicity assessment included: acute tests with *A. bahia* and *M. beryllina*, the freshwater crustacean *Ceriodaphina dubia*, and the freshwater fish *Pimephales promelas*; development of the echinoderm *Arbacia punctulata*; and growth of a freshwater (*Raphidocelis subcapitata*) and marine (*Dunaliella tertiolecta*) algae. Siltech acute toxicity values ranged between 1.1 ppm to 32.8 ppm, with *A. punctulata* being the most sensitive and *R. subcapitata* the least. ThickSlick acute toxicity values ranged from 2.2 ppm to 126.4 ppm, with *C. dubia* being the most sensitive and *M. beryllina* the least. The results of this study show generally greater toxicity of Siltech compared to Thickslick and provides for a greater understanding of species sensitivity of these two oil spill response agents. While it is unlikely that a herding agent will be used in the absence of spilled oil, evaluating the toxicity of agent alone is a preliminary step that will help improve oil spill response protocols and help minimize the environmental impacts of oil spills or response operations.

02.07 Ecotoxicology in a Rapidly Changing Climate: Multiple Stressor Effects on Aquatic Organisms in Climate Change Scenario

02.07.01 Does Adaptation to Warmer Temperatures Impact Susceptibility to Pesticides?

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The objective of this study was to investigate how adaptation to increased temperature might cause organisms to be more susceptible to toxic substances. Chlorpyrifos is a highly metabolized organophosphate insecticide that must be bioactivated into the toxic metabolite, chlorpyrifos-oxon, in order to elicit toxic effects. Using two genotypes of *Daphnia pulex* as the model organism, the toxicity of both chlorpyrifos and chlorpyrifos-oxon was tested. The two genotypes were lab raised and differ in their genotype for a gene called *Pgi*. *Pgi* is involved in glycolysis, impacting the metabolic rate of the organism, and is also temperature sensitive. It is therefore reasonable to hypothesize that the two populations that differ in their genotypes for the gene *Pgi* will 1) differ in their susceptibility to chlorpyrifos, a pesticide that is highly metabolized, and 2) differ in the degree that temperature impacts chlorpyrifos toxicity. Following acute toxicity tests for chlorpyrifos and chlorpyrifos-oxon at warm and cold temperatures, EC50s were calculated and compared between the two *Pgi* genotypes. Only small susceptibility differences were found for chlorpyrifos between the two genotypes, and no significant differences were found for chlorpyrifos-oxon. Temperature treatment (warm vs cold) and concentration of chemical were both significant factors influencing toxicity of both chemicals (p-values < 0.01).

02.07.02 Influence of Salinity on Pyrethroid Toxicity: Behavior in a Model Estuarine Organism (Menidia beryllina)

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Climate change is causing changes in precipitation patterns and contributing to increased sea levels. These alterations are linked with changes in salinity in estuaries, making the differences in toxicity across a salinity gradient a topic of increasing interest in assessing risk to estuarine fish species. Several recent studies have shown that pyrethroid toxicity can change across a salinity gradient. Pyrethroid pesticides are commonly used globally in agricultural, industrial, and household settings. Behavior has been demonstrated to be a sensitive endpoint and can detect the toxic effects of environmentally relevant concentrations of aquatic pollutants. Early life exposures in fish to pyrethroids has been found to cause toxicity at environmentally relevant concentrations, as well as to alter behavior. Inland Silversides (*Menidia beryllina*) are a commonly used euryhaline, model fish species and are approved by the United States Environmental Protection Agency for estuarine and marine toxicity testing. Therefore, Silversides were exposed from 5 days post fertilization (approximately 1-day pre-hatch) for 96 hours to six different pyrethroids for which freshwater safety thresholds have been determined in the State of California: bifenthrin, cyfluthrin, cyhalothrin, permethrin, cypermethrin, and esfenvalerate. Exposures were conducted at three salinities relevant to brackish, estuarine habitat (0.5, 2, and 6 PSU) and 3 concentrations, either 0.1, 1, 10, and/or 100 ng/L, determined from previous experiments to be sublethal and environmentally relevant. After exposure, Silversides underwent behavioral assays and were subjected to a dark and light cycle, as well as a tap stimulus, to determine toxicity. Changes in behavior were analyzed between salinity and chemical gradients. Results show significant behavioral changes with pyrethroid exposure at all three salinities and concentrations. Additionally, these results indicate that there may be different behavioral responses to pyrethroids depending on pyrethroid type and salinity. These data will provide knowledge to managers and environmental planners to help further protect threatened and endangered fishes in estuarine and bay regions.

02.07.03 Effects of Dietary Pesticide Mixtures and Temperature on the Swimming Performance of Juvenile Chinook Salmon (Oncorhynchus tshawytscha)

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Studies previously conducted to understand the toxic potential of pesticides to aquatic biota have been predominantly based on waterborne exposures, not addressing other routes of uptake, which would provide new insights when making management decisions. As such, the toxicity of pesticides from dietary uptake are largely unknown in fish. This is of particular concern to endangered fish species, such as the Chinook salmon, that have experienced large reductions in spawning numbers in the Sacramento-San Joaquin Delta over the last decade. Predicted increases in the frequency of droughts within the Delta due to climate change, likely warming water temperatures in habitats used by Chinook, further supports the need to understand the role of a changing climate to pesticide toxicity. To evaluate the impact of temperature on the dietary uptake of pesticides found within the Delta and the potential adverse effects to salmonids, juvenile Chinook were dietarily exposed to a mixture of current, restricted-use, and a degradate of a legacy pesticide based on identified pesticide classes and measured concentrations from field-collected invertebrates in the Delta. A representative low (102 ng/g DDE, 35 ng/g bifenthrin, 5 ng/g chlorpyrifos, 12 ng/g esfenvalerate, and 88 ng/g fipronil) and high (610 ng/g DDE, 212 ng/g bifenthrin, 40 ng/g chlorpyrifos, 70 ng/g esfenvalerate, and 527 ng/g fipronil) pesticide mixture was fed to juvenile Chinook for two weeks under three temperature scenarios, 11, 14, and 17°C. To understand the sublethal effects of pesticide mixtures and influence of temperature on toxicity, burst swimming speed (U_{max}) was used to assess swimming performance following treatment, with liver samples subsequently collected for gene expression, targeting genes important in lipid metabolism and energetics. Chinook salmon dietarily exposed to the low and high mixture at 17°C had a significantly reduced U_{max}, relative to controls, with a dysregulation in ATP citrate synthase expression. This data suggests that juvenile Chinook dietarily exposed to concentrations of pesticides measured within the field under increased water temperatures, predicted from climate models, impair swimming performance, and potentially alters metabolic pathways, suggesting effects in foraging ability and predation for juvenile Chinook in rearing habitats in the Delta.

02.07.04 Effect of Terrestrial and Aquatic Dissolved Organic Matter on Uptake Rates of Teflubenzuron in the Tunicate Ciona intestinalis

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In recent years a darkening of aquatic environments has been observed and traced back to an increased output of terrestrial dissolved organic matter (tDOM). Dissolved organic matter can be derived from the terrestrial environment (tDOM) or from the aquatic environment (aDOM). Due to the aromatic nature, large size, and higher biorefractory nature of the terrestrial DOM humic acids compared to aquatic DOM, which is lighter and with smaller molecules, terrestrial DOM has a substantial effect on the physicochemical conditions of the water column in marine environments. The presence of a larger tDOM molecular structure is hypothesized to cause higher adsorption of lipophilic contaminants to terrestrial DOM, and if this bioaccumulates in the organism at a low trophic level it can transport up the food web and thus impact the whole system. Previously, we have shown that Teflubenzuron uptake in a filter feeder (blue mussel) is affected by the DOM origin. Teflubenzuron, belongs to a group of insecticides highly used in aquaculture along the coast against sea lice on salmon. Teflubenzuron is highly toxic to aquatic invertebrates and nontarget species in the vicinity of the salmon farms may be exposed to this contaminant. This study addresses how the type of dissolved organic matter, aquatic DOM vs terrestrial DOM, impact contaminant patterns in ascidians, more specifically adult *Ciona intestinalis* which is a common sessile filter feeder. Compared to the blue mussels, ascidians have higher filter efficiency and a potential target for Teflubenzuron exposure. In particular, it addresses whether the terrestrial derived DOM or the aquatic derived DOM acts as a stronger food vector under high DOM conditions. The experiment consists of four DOM exposure treatments: tDOM, aDOM, a mixture of aDOM and tDOM, and seawater. The organisms are randomly distributed into 24 aquaria for a 16-day exposure. In addition to the DOM exposure treatments, half of the aquaria are treated with Teflubenzuron and the other half receives the DOM exposure treatments. One individual was sampled from each aquarium every three days. We hypothesize that tDOM is a better vector for lipophilic contaminants than aDOM in *C. intestinalis* and expects higher bioaccumulation of Teflubenzuron in the organisms that are exposed to tDOM, which may lead to increased biomagnification.

02.07.05 Effect of Terrestrial and Aquatic Dissolved Organic Matter on Uptake Rates of Teflubenzuron in Blue Mussels (Mytilus edulis)

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Coastal darkening, linked to ongoing increases in inputs of terrestrial dissolved organic matter (DOM) to coastal waters, has been observed in several recent studies and caused by ongoing environmental changes, including climate change. The darkening is driven by qualitative changes in coastal DOM, with an increasing contribution of highly-colored (and high molecular weight) terrestrially-derived DOM (tDOM) in coastal waters where less-coloured (and lower molecular weight) DOM of aquatic (marine phytoplankton) origin (aDOM) previously prevailed. Due to the higher molecular weight of tDOM and its “enveloping” properties, it can be hypothesized that tDOM is an efficient food vector for lipophilic contaminants. Previous studies show that the presence of tDOM can lead to reduced concentration of contaminants in the open water column and less uptake directly from the water into organisms (bioconcentration). However, the very same properties may lead to an increased contaminant uptake via feeding processes, especially in filter feeding organisms leading to increased concentrations at higher trophic levels through biomagnification. To assess changes in contaminant uptake, we exposed 312 blue mussels (Mytilus edulis) in a factorial design that was comprised of 24 aquaria with four different DOM conditions in the presence or absence of teflubenzuron. Teflubenzuron is a lipophilic (log KOW: 5.39) veterinary drug commonly used in aquaculture against sea lice infestations. The different DOM treatments included aquatic DOM derived from phytoplankton, terrestrial DOM derived from forest leaves, a mix of the two, and seawater with low natural DOM as control. The experiment was run with three weeks acclimation, two weeks exposure phase and three weeks depuration phase. Teflubenzuron concentrations in tissue and water were subsequently measured and compared between the different treatments.

Against expectation, higher in-tissue concentration of the contaminant was found in the mussels exposed to the mixture DOM, followed by the aDOM, tDOM and the low DOM treatment. Possible reasons for this may be higher bioavailability of aquatic DOM for bacteria and the mussels' food size preference for bacteria sized particles. This is further addressed in a follow-up experiment with ascidians (separate presentation). These studies combined give important insights into contaminant transfer under changing DOM conditions with implications for different members of the coastal food webs.

02.07.06 Effects of Environmentally Relevant Micro and Nano Plastics (Polypropylene, Polylactic Acid and Polyethylene Terephthalate) Particles on Estuarine Indicator Species

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Since the 1950s, plastics have gradually overtaken the global market, with a predicted exponential increase in production and disposal out to 2050. These trends have led to increasing microplastic (< 5 mm) concentrations in estuaries and coastal waters. With time plastics undergo various physical changes and wear down resulting in micro and nano size plastic particles (MNPs) and mix into marine debris and could potentially take up by marine biota and potentially cause adverse health impacts. Internalization of and interactions with MNPs may cause potential adverse health impacts on aquatic biota. In this study polypropylene (PP), polylactic acids (PLA) and Polyethylene terephthalate (PETE) created from single use plastics (e.g., water bottle, straws) were used in exposures to represent marine debris at micro and nano size, and to also account for bioplastics (PLA), which are increasing in production and use. The estuarine indicator fish *Menidia beryllina* and invertebrate *Americanmysis bahia* were exposed at three salinities (5, 15, 25 ppt for fish / 15, 20, 25 ppt for mysid shrimp). We conducted an assessment of growth and behavior, endpoints which are shown to be highly sensitive to micro and nanoplastic exposure. Results indicates significant differences in behavior at the highest and lowest salinities for silverside fish (*Menidia beryllina)* and mysid shrimp (A*mericamysis bahia*) (1 day pre-hatch – 4 days post hatch; 7-14 days post hatch, respectively). These results can fill in gaps for potential impacts of plastic debris in estuarine organisms, similar to what findings in other studies. These data will be used in a risk assessment framework for aquatic organisms' exposure to environmentally relevant concentrations across a wide range of salinities and other conditions.

02.07.07 Transgenerational Effects of Warming, Ocean Acidification and Polycyclic Aromatic Hydrocarbons on a Key Arctic Copepod Calanus glacialis

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In the Arctic marine ecosystems, *Calanus glacialis* is a key species, linking primary and secondary producers, as well as having a large amount of biomass, and containing lipids required by many organisms at higher trophic levels. Arctic marine ecosystems are under threat from multiple stressors, particularly climate change, ocean acidification (OA), and pollution such as polycyclic aromatic hydrocarbons (PAHs) from increased shipping and oil exploitation. We know very little about how these stressors may affect *C. glacialis*.Recent advancements in evolutionary ecotoxicology show that phenotypic plasticity allows for adaptation to changes in the environment, and this can occur across generations when conditions experienced by previous generations influence the traits of the offspring. However, *C. glacialis* is highly specialized to Arctic conditions, which may limit their transgenerational plasticity to single and multiple stressors, but this novel hypothesis has not been tested. In this study, we investigated whether parental exposures to warming (1 and 5°C), OA (pH 8.05 and 7.55) and pyrene (0 and 200 nM) may affect offspring fitness. Eggs from stressor-exposed females were collected and incubated in the clean environment at control temperature and pH. The hatching success, morphological changes and development will be tracked for all nauplii stages (N1-N6). This ongoing experiment is planned to complete by the end of June 2021 and results will be analyzed and updated by November 2021.

02.07.08 Thermal Modulation of Mitochondrial Function Is Affected by Nickel in Rainbow Trout (Oncorhynchus mykiss)

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In a world affected by global changes and anthropogenic activities, aquatic organisms are chronically exposed to multiple sources of stressors. In this study, we investigated the combined effects of temperature and nickel (Ni) contamination on liver mitochondria electron transport system (ETS) enzymes, citrate synthase (CS), phospholipid fatty acid composition and lipid peroxidation (LPO) in rainbow trout (*Oncorhynchus mykiss*). Juvenile trout were acclimated for two weeks to one of two temperatures (5°C or 15°C) and exposed to Ni (520 µg/L) for three weeks. Using ratios of ETS enzymes and CS activities, our data suggests a synergistic effect of Ni and temperature yielding a higher reduction status of the ETS. The response of phospholipid fatty acid profiles to thermal variation was altered under Ni exposure. In control conditions, the proportion of saturated fatty acids (SFA) was higher at 15°C than at 5°C, while the opposite was observed for monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA). In contrast, in Ni contaminated fish, the proportion of SFA was higher at 5°C than at 15°C, while PUFA and MUFA followed the opposite direction. A higher PUFA ratio is associated with higher vulnerability to LPO. Thiobarbituric Acid Reactive Substances (TBARS) content, an indicator of LPO, varied accordingly, except for warm-acclimated fish under Ni exposure, for which we reported the lowest level of TBARS, suggesting an interaction of Ni and temperature affecting both antioxidant capacities and aerobic energy metabolism. Data from our study suggest that the combined variations of complexes I and IV of ETS and CS caused by Ni at higher temperature could stimulate multiple sources of ROS production while the relative decrease of complex II could counteract to compensate this overproduction. These results suggest that high temperature and Ni exposure combined can lead to an increase of metabolic demand, caused by the remodelling of the mitochondrial phenotype and alternative antioxidant mechanisms.Nevertheless, the higher metabolic cost begotten can affect fish condition under longer exposure. Combined effects of Ni exposure and temperature augmentation lead to the remodulation of the ETS, in concordance with oxidative stress markers, independently of single stress exposure. These results highlight the importance of mitochondrial phenotype flexibility to respond to these environmental stressors, in a context of growing mining activity and climate change.

02.07.09 Elevated Temperatures Exacerbate the Neurotoxic Effects of Dietary Exposure to Chlorpyrifos in Inland Silversides, Menidia beryllina

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Chlorpyrifos, an organophosphate (OP) insecticide, is prevalent in aquatic systems globally and is often implicated in aquatic toxicity during storm events. Chlorpyrifos induces toxicity by inhibition of acetylcholinesterase

(AChE) activity, which has been related to alterations to swimming performance in fish. Resistance to organophosphate insecticides, including chlorpyrifos, is prevalent in populations of the epibenthic amphipod *Hyalella azteca* in areas with known OP exposure. Previous studies have demonstrated an elevated bioaccumulation potential of insecticide-resistant prey items, however the potential for trophic transfer of chlorpyrifos from OP-resistant prey items and associated neurotoxic effects in fish predators has not been studied. Concurrent with dietary exposure, global climate change is anticipated to significantly alter abiotic parameters in aquatic systems, including temperature. Furthermore, previous studies have demonstrated the potential for temperature to exacerbate the neurotoxic effects of organophosphate insecticides. Consequently, the present study aimed to determine the trophic transfer and effects of chlorpyrifos from OP-resistant *H. azteca* to a known predator, the inland silverside, *Menidia beryllina* at two temperatures (18 and 23°C). Fish were fed either ^{14}C-chlorpyrifos-dosed *H. azteca* or control animals for 7 d, after which total bioaccumulation, percent parent chlorpyrifos, brain AChE activity and swimming performance (ramp-Ucrit) was determined. Fish fed chlorpyrifos-dosed *H. azteca* bioaccumulated significant amounts of chlorpyrifos, ranging from 29.9 to 1250 ng/g lipid, demonstrating the potential for trophic transfer. A significant reduction in brain AChE activity was observed in fish fed chlorpyrifos-dosed *H. azteca* at 23°C only, which may be attributed to increased biotransformation of parent chlorpyrifos to more potent AChE-inhibiting metabolites. Dietary chlorpyrifos exposure had no significant effect on swimming performance in *M. beryllina*, though ramp-Ucrit was significantly increased at 23 compared to 18°C. These findings confirm the potential for trophic transfer of chlorpyrifos from OP-resistant prey items to fish predators and the potential for elevated temperatures to exacerbate the neurotoxic effects of chlorpyrifos.

02.07.10 Thiamine Deficiency as an Ecological Stressor Driven by Anthropogenic Nutrient Cycle Changes, Invasive Species, and Global Climate Change

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Thiamine (vitamin B_1) deficiency is an emerging global challenge for ecological health. Thiamine is an essential co-factor for energy metabolism enzymes and is required by all organisms. Some organisms, including species of bacteria, ferns, mollusks, and fish, contain thiamine-degrading enzymes known as thiaminases, and consumption of these organisms can lead to thiamine deficiency complex (TDC) in the consumer. Effects of TDC may mimic and/or interact with effects of other stressors such as diseases and contaminant exposures. Ecosystems around the world have shown evidence of TDC in birds, fish, and wildlife, often correlated with anthropogenic changes such as food web alterations, invasive species, and global climate change. In the Baltic Sea, TDC has been implicated in spawning migration failures in Atlantic salmon. In the Great Lakes, low thiamine leads to elevated early life stage mortality prior to initiation of exogenous feeding in native lake trout (*Salvelinus namaycush*) populations that consume the non-native, thiaminase-carrying prey fish alewife (*Alosa pseudoharengus*). On the North American Pacific coast, native salmonid populations have shown signs of TDC associated with changes in the marine food web, likely related to record high ocean temperatures. Specifically, TDC in California salmon appears to be linked with reduced diversity and dominance of thiaminase-carrying northern anchovy (*Engraulis mordax*), in salmon diets. We present an overview of our current knowledge of potential causes of TDC, evidence for de novo production of thiaminase by some species of prey fish, and results of monitoring thiamine levels in salmonids from several populations in North America.

02.07.11 Coupled Hydrologic, Chemical Fate, and Uptake Models for Predicting Current and Future Contaminant Trends in the South Saskatchewan River

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Global changes in climate have been observed over the past decades, which lead to varied conditions in many environments, including freshwater ecosystems. These variations, in turn, also impact the processes by which chemical contaminants move through aquatic environments. These chemicals are eventually taken up by aquatic organisms, where they can cause harmful effects. However, knowledge gaps related to the impacts of climate change on the underlying hydrological, chemical, and biological processes currently limit our ability to forecast potential future changes in contaminant concentrations accurately. This research aims to combine computer models for the prediction of hydrologic processes, chemical fate, and uptake of chemicals into aquatic organisms. The study site for this research will be the hydrologically well-characterized South Saskatchewan River in Saskatchewan, which is one of the most important water resources in Western Canada. Along this area, high-quality data on the concentration of specific contaminants in water, suspended particles, and sediments are currently lacking and will be generated through chemical analysis to develop and test these models. Fish species native to the area will also be collected and analyzed for chemical concentrations to understand the contaminant uptake. Results obtained from the sample analyses will provide useful baseline contaminant profiles for the South Saskatchewan River. The coupled model will be a highly valuable tool for the prediction of the impacts of climate change on contaminant movement through the South Saskatchewan River. It would benefit Canadians, especially Indigenous communities who rely on the river for food and water, in helping them to adapt to or mitigate future climate change-related water threats. This study could also serve as a blueprint for other river systems around the world.

02.08 Ecotoxicology in Tropical Ecosystems

02.08.02 Systematic Evidence Maps Relating Water Quality Thresholds to Coral Reef Health

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Water quality stressors such as nutrients, sediments, temperature, dissolved oxygen, metals and others are known to have deleterious effects on a variety of individual and population scale coral reef endpoints. U.S. coral reef jurisdictions have promulgated a variety of water quality standards for these and other water quality stressors; however, most of these standards are not crafted specifically for coral reef endpoints, as data gaps remain in understanding which stressor levels are protective of which types of coral reef endpoints. To facilitate management of the impacts of water quality on coral reef ecosystems, this project develops a methodical compilation of the existing body of information relating water quality measurements to coral reef ecosystem condition. The information is presented as a systematic evidence map, which is a summary of the kind of scientific findings available to answer a defined set of policy or management questions. The goal of this systematic map is to comprehensively organize the available literature on a specific set of select water quality and coral reef metrics and build out an evidence base of data availability and type. The evidence base is guided by a conceptual model identifying causal relationships between water quality stressors and coral reef endpoints, in the context of other stressors, location differences, climate interactions, and land management choices. The compiled and screened evidence base is then mapped to the conceptual model to understand: which causal relationships have a lot versus a little evidence; type and strength of existing evidence; magnitude and consistency of links reported under what conditions; synergies of multiple stressors or conditions; and areas with information gaps. Study titles and abstracts are reviewed for relevance and to extract initial information on a suite of water quality factors affecting coral reef endpoints at the qualitative level, to get a sense of how much and what kind of literature exists for each of the water quality stressors of import in the coral reef conceptual model. Results could be used to rank stressors, understand the interactions among stressors, and inform the circumstances under which location affects stressors. This evidence mapping approach helps clarify the types of decisions that need to be made by jurisdictions to develop coral reef-specific water quality standards or other types of water quality management methods.

02.08.03 Mercury Accumulation in Tropical Estuarine Fishes, Colombian Pacific

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Mercury in estuarine fishes is a problem that has been increasing, despite many efforts to reduce mercury emissions. The objective of this study was to determine the accumulation of mercury in multiple species of tropical estuarine fish, with Buenaventura Bay as a model estuary, generating a baseline of information on little-studied species. Fish samples were collected from different areas and hydroclimatic seasons, representing the spatiotemporal gradients of the estuary, and the total mercury concentration (THg, dry weight) in fish muscle was determined. Total mercury content (dry weight) was analyzed in the muscle of 2,127 fish from 67 species, 50 genera, and 28 families in the Buenaventura Bay estuary. All the studied species presented THg in their tissues. THg concentrations >0.2 $\mu g\ g^{-1}$detected in >50% of the analyzed species. The species with greatest THg concentration was from the family Scianidae, among which *Larimus argenteus* (mean ± SD; 1.431 ± 0.978 $\mu g\ g^{-1}$) presented concentrations 2.5 times higher than the next species with the highest THg content, *Stellifer mancorensis* (0.574 ± 0.212 $\mu g\ g^{-1}$). The individual that showed the greatest THg content (2.8676 $\mu g\ g^{-1}$) was a *L. argenteus* specimen, measuring 35.5 cm in TL and weighing 475 g, captured during the dry season in the inner part of the estuary. This results suggest that mercury bioaccumulation occurs in the Buenaventura Bay estuary.

02.08.05 Effect of Microplastic Pollution on the Mesoamerican Reef

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In the Mexican Caribbean, the Mesoamerican Reef System (SAM) is located, the second largest reef system in the world, which extends from Contoy Island in the north of the Yucatan Peninsula to Honduras. Coral reefs represent an important resource from an economic, fishing and tourist point of view. The conservation of these ecosystems is of fundamental importance due to their great biodiversity, high productivity, richness and abundance of species. These environments are now also exposed to microplastics being complex and diverse pollutants of great interest due to their potential effects due to their persistence, ubiquity and diversity of size-type plastic polymers. The objective of this work is to carry out a review of the effects of microplastics in the Mexican Caribbean, identifying the possible damages on these organisms, among which whitening, mucus production, tissue necrosis and death of the organism have been observed, on the other hand in the symbiont it increases adhesion and promotes photosynthetic efficiency. The high use of plastics has been shown to harm the environment and organisms. Thus, serious pollution problems can be generated, which is why more extensive studies have to be carried out in the reef areas for their protection and to generate protection measures for these ecosystems.

02.09 Fate and Effects of Chemicals from Stormwater Runoff

02.09.01 Contaminants of Emerging Concern in Urban Stormwater and San Francisco Bay

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San Francisco Bay is the largest estuary on the west coast of the Americas and supports an urban population of over 7 million people. The region serves as a useful laboratory for studies of anthropogenic contaminants in urban stormwater, as well as impacts on receiving waters. Urban tributaries contribute a large percentage of the pollutant load to the Bay for many legacy contaminants, including polychlorinated biphenyls (PCBs), dioxins, polycyclic aromatic hydrocarbons (PAHs), and trace metals, despite supplying just 6% of the freshwater entering the Bay. However, far less is known about the role of urban stormwater in delivering contaminants of emerging concern (CECs) to the Bay, a data gap generally observed in receiving waters globally. To begin to address this data gap, the Regional Monitoring Program for Water Quality in San Francisco Bay (RMP) is conducting a four-year screening study to evaluate the concentrations of key classes of CECs in stormwater. Sampling sites have been selected primarily based on the extent of upstream urban land uses, with an emphasis on proximity to roadways and unique land uses associated with potential contaminant sources. Five classes of compounds are being monitored, including per- and polyfluoroalkyl substances (PFAS), ethoxylated surfactants, organophosphate esters (OPEs), bisphenols, and several chemicals associated specifically with urban stormwater, such as urban use pesticides and ingredients in vehicle tires. Results from the first three years of sampling will be summarized; sampling will continue through 2022. Initial findings have informed development of a new study to quantify stormwater-delivered contaminants in Bay water during the wet season (November 2021 – April 2022), when this contaminant pathway is active.

02.09.02 Occurrence and Distribution of Pyrethroid Insecticides in California Urban Storm Drain Systems

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Pyrethroids are some of the most commonly used insecticides, with widespread use in agricultural and urban settings. Urban waterways are frequently contaminated with pyrethroids, with sources such as residential use believed to be significant contributors. Many studies have monitored pyrethroid occurrence in neighborhoods and downstream of outfalls, but little work has been conducted on the urban storm drainage systems which divert stormwater runoff. Media in these systems may frequently contain pyrethroids and act as a sink for residues. The current study sought to measure pyrethroid concentrations in environmental phases, such as water, sediment, and biofilms, across California urban drainage structures, such as catch basins, flood channels, and outfalls. Findings from this work will help clarify urban pyrethroid transport and elucidate risks, such as non-target aquatic toxicity and insecticide resistance selection pressure.

02.09.03 Occurrences of Tire Rubber Derived Contaminants in Cold-Climate Urban Runoff

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Recent findings that the quinone transformation product of a common tire rubber antioxidant (N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine, 6PPD) is ubiquitous and acutely toxic in stormwater impacted streams has highlighted the need for a better understanding of contaminants related to tire rubber in urban runoff. This study represents the first report of 6PPD-quinone and other tire rubber-related compounds in stormwater and snowmelt of a cold-climate Canadian city. Snowmelt and stormwater were sampled across the City of Saskatoon in 2019 and 2020. Of the five target compounds, the common rubber vulcanizing agent N,N'-diphenylguanidine (DPG) was most abundant, with average concentrations of 60 µg L^{-1} in stormwater and 1 µg L^{-1} in snowmelt. Peak concentrations of DPG reached >300 µg L^{-1}, equivalent to loadings of >15 kg from a single rain event. These concentrations of DPG represent some of the highest reported in urban runoff globally. 6PPD-quinone was detected in 57% of stormwater samples with a mean concentration of ≈600 ng L^{-1} (2019) and in >80% of snowmelt samples with mean concentrations of 80 – 370 ng L^{-1} (2019 and 2020). Concentrations of 6PPD-quinone exceeded the acute LC_{50} for coho salmon (0.8 – 1.2 µg L^{-1}) in >20% of all stormwater samples. This study confirms the widespread occurrence of tire rubber-related compounds in urban runoff and highlights the urgent need to characterize the ecological risks of these compounds on impacted aquatic systems.

02.09.04 Particle Size Characterization and Fate of Per- and Polyfluorinated Substances in Stormwater

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Per- and polyfluorinated substances (PFAS) are a group of manmade compounds that have been widely used in applications such as non-stick cookware and fire fighting foams but are increasingly the focus of environmental concerns. Relative to other classes of contaminants, PFAS exhibit weak sorption, low retardation, and high mobility, which has contributed to concerns regarding their occurrence in aqueous systems. Their occurrence in groundwater and surface waters has been established, but studies investigating PFAS occurrence and mitigation in stormwater systems are only just emerging. In this study we seek to better understand the behavior of PFAS in stormwater and the ability of common stormwater management systems to mitigate PFAS release. PFAS contamination has been detected at Former Reese Air Force Base (now known as Reese Technology Center (RTC), Lubbock TX), as a result of the use of fire fighting foams. Stormwater was collected using automated samplers from 3 different outfalls that discharge into a retention pond as a conventional and common strategy to manage stormwater releases. Retention Pond water and sediment samples were also collected before and after storm events. The collected stormwater samples were size fractioned to determine the concentration associated with different particle fractions (broadly classified as clay, silt and sand) as well as in dissolved fractions. The samples were analyzed for PFAS via high performance liquid chromatography (HPLC) coupled with a Sciex X500R quadrupole time of flight mass spectrometer (QToF). Preliminary results suggest that essentially all of the PFAS is associated with the dissolved fraction of stormwater with the exception of stronger sorbing PFAS (e.g., PFOS, L_PFOS) which had significant association with stormwater solids. Since the primary role of a retention pond is to allow settling time for solids, its effectiveness for most of the PFAS was minimal. The concentrations of PFAS in the water column of the retention pond, and ultimately discharged from the retention pond, were essentially unchanged from that expected stormwater inputs.

02.09.05 Urban Drool: Dry-Weather Water Quality in Denver, Colorado: Relationships Between Urban Catchment Characteristics and Pollutant Loads

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Though stormwater is a significant contributing factor to urban surface water pollution, dry-weather pollutant loads also represent an important source of contamination, particularly in semi-arid urban environments, as they are strictly correlated to groundwater contributions. Dry-weather water quality data is much less abundant than stormwater, yet several past dry-weather studies in urban catchments demonstrated that dry-weather pollutant loads may be greater than stormwater nutrient, pathogen, and select metal loads during notably dry weather periods. Denver, Colorado represents an expanding urban area in a semi-arid environment that is an ideal area to investigate dry-weather surface-water quality. The purpose of this study is to identify relationships between urban catchment characteristics (e.g. various land uses, traffic, and impervious area) and dry-weather water quality. In this study, water samples are taken throughout Denver at urban drainage points (e.g. gulches, outfalls, and other urban drainage that maintain year-round flows) along the South Platte River and are assessed for various water quality parameters, including total suspended solids, coliforms, *E. Coli*, nutrients, dissolved and total organic carbon, and dissolved and total recoverable metals. Sampling points in Denver were determined using previously collected data from the City of Denver, ArcGIS, and Google Earth to obtain various catchment drainage points representing a variety of urban characteristics, ranging from residential single-family homes to dense infill urban areas. Preliminary results show an increase in nutrient concentration (nitrogen, phosphorus, and potassium) in watersheds with more impervious surfaces and urban land uses, which is counter intuitive. Selenium and nitrate were also observed at concentrations above compliance standards and are likely linked to groundwater infiltration to urban water channels. Further results will be identified as data analysis is currently underway, where the study should be finished by the end of Summer 2021. This information will not only alert the City of Denver to what catchment areas are out of compliance, but it will also facilitate and inform dry-weather pollution mitigation decisions that would benefit and improve urban stream water quality.

02.09.07 Environmental Impacts of the Application of Potassium Acetate as a Deicer

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The adverse effects of the use of chloride-based salt as a deicer in winter road maintenance to water quality, aquatic ecosystems, and infrastructure are driving a search for effective alternatives to chloride-based salt. Potassium acetate (KAc) is a biodegradable liquid deicer shown to be effective at lower temperatures and less corrosive than chloride-based deicers. However, the environmental impacts of KAc application are not fully understood. Due to the organic nature of acetate-based deicers, KAc application may exert a biological oxygen demand in water bodies receiving stormwater carrying KAc. Additionally, the application of KAc will result in increasing short- and long-term concentrations of K^+ and Ac^- in water bodies that may reach toxic levels to sensitive species. CF-7 (the

KAc commercial product) has been applied on select roadways in Duluth, Minnesota located in Lake Superior watershed as a preliminary field evaluation. We conducted an environmental field evaluation to determine ionic concentrations with water quality parameters in stormwater and the receiving water body, Lake Superior. Water samples were collected with a combination of automated samplers and grab sampling following snow events during the winters of 2019-2021. Major ions, dissolved organic carbon, metals, BOD_5, and *E. coli*. were analyzed to evaluate the persistence and impact of KAc. Biodegradation experiments of CF7 were also conducted to determine the degradation rate of acetate in Lake Superior water under different temperatures mimicking the fate of acetate in the field. The concentrations of KAc deicers were found to be at levels causing lethal and sublethal effects to roadside vegetation and aquatic organisms. KAc application appears to influence BOD_5 and microbiological water quality depending on characteristics of receiving water such as lake volume, mixing, and wind. Understanding the concentration of K^+ and $Ac^-$with other water chemistry in the highway or bridge runoff and receiving water through this field evaluation will be useful for modeling the watershed impacts of KAc use as a road salt alternative.

02.09.08 Toxicity Evaluation of Potassium Acetate as a Road Salt Alternative

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Chloride-based salts have been used as an effective snow and ice management technique for winter roads, parking lots, and sidewalks. Despite effective for deicing, the chloride-based salts are corrosive to infrastructure, elevate chloride levels in nearby water bodies, and affect the breakdown of soil. To improve water quality and mitigate the overall cost of road salt, an effective alternative to chloride-based deicing material is needed. One alternative that has potential is potassium acetate (KAc), which is effective at lower temperatures than chloride-based salts and is much less corrosive to steel. CF7®by Cytotech is one acetate-based deicing formulation that contains ~50 percent potassium acetate that is being considered for application in the state of Minnesota. Prior to the decision, it is essential to assess the environmental effects of KAc, as an acetate-based deicer potentially exerts a biochemical oxygen demand (BOD) and contribute ionic toxicity to aquatic organisms. Laboratory experiments were conducted to assess the effect of KAc and CF7®(a commercial KAc deicer) on roadside plant seed germination and aquatic toxicity tests were performed to assess the toxicity of formulated CF7®attributable to KAc (versus other additives) and to determine the toxicity of the acetate. These tests included acute 48-hour tests with the commercial KAc deicer, Cryotech CF7®, potassium acetate (KAc), and sodium acetate (NaAc) with two species of cladocerans, *Ceriodaphnia dubia* and *Daphnia magna* to assess the role of acetate in the toxicity. The results of the acute toxicity tests with both cladocerans were similar those of the previoUSEPA data on the acute toxicity of major ion salts. The seed germination experiments were conducted with regional roadside plants with concentration levels found in stormwater and receiving water and the accumulation of ionic accumulation on the root was examined by Scanning Electron Microscopy. The toxicity results gained from this work will inform decision makers on the environmental impact of using KAc as a road salt alternative along with field evaluation of KAc concentration in stormwater and receiving water. Disclaimer: The views expressed in this presentation are those of the authors and do not necessarily represent the views or policies of the Agency.

02.09.09 Water Quality Impacts to Lake Elsinore, CA From Stormwater Runoff Following the 2018 Holy Fire

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The Holy Fire wildfire in Southern California began on August 6, 2018 and reached full containment on September 13, 2018 with a total burned area of 35.9 square miles. Lake Elsinore lies at the bottom of several large canyons draining the burned area. Since the fire containment, the area received numerous storms of varying strength from < 0.25 to 5.5 inches, totaling approximately 25 inches of rain from October 2018 through March 2019. Debris flows from the burn area deposited a large sediment delta in the lake. Shortly after the first two major storms in late November 2018 hit the area, a large fish die-off was observed in Lake Elsinore which continued through January 2019. Based on multiple lines of evidence this die-off was attributed to the Golden Algae, *Prymnesium parvum*, a species not previously observed at high densities in the lake. The City of Lake Elsinore embarked on a study to determine if runoff from the Holy Fire had triggered the Golden Algae bloom and subsequent fish kill. Chemistry and toxicity analyses were performed on both water and sediment samples in and around the fire sediment delta. Samples for phytoplankton taxonomy were also collected in and around the fire sediment delta, as well as during monthly routine mid-lake sampling. A comprehensive analysis of results will be presented showing the magnitude of effect on lake water and sediment quality and potential for long-term impacts.

02.09.10 Blood-Brain Barrier Disruption in Juvenile Coho Salmon Exposed to Roadway Runoff

S. Blair, Washington State University / Puyallup Research and Extension Center; C. Barlow, Evergreen State College; J. McIntyre, Washington State University, Puyallup / School of the Environment

Urban stormwater pollution is a rapidly rising global threat to biodiversity and human health. In the Pacific Northwest of North America, coho salmon (*Oncorhynchus kisutch*) are a sentinel species for urban runoff pollution. Persistent high rates of coho salmon prespawn mortality in urban streams are driven by the ubiquitous tire chemical 6PPD-quinone. As a newly discovered environmental contaminant, information is needed on the toxic mechanisms of action of 6PPD-quinone to explain the lethal effects seen in coho and to explore potential sublethal effects in other species. In previous studies, we demonstrated that exposure to roadway runoff causes severe increases in hematocrit and plasma leakage concurrent with the development of loss-of-equilibrium in coho, indicating for blood-brain barrier disruption as the primary mode of action. We provide additional evidence of the presence of blood-brain barrier disruption based on histological and quantitative methods using a several high and low molecular weight tracers. Understanding the mode of action in coho urban runoff mortality syndrome is important to evaluate sublethal effects due to runoff exposure for a variety of aquatic species of concern in urbanizing environments, such as threatened Puget Sound Chinook salmon (*O. tshawytscha*).

02.09.11 Biogeochemical Responses to Changing Salinity Regimes in Northwestern Nevada River Basins

K. Seto, J.R. Blaszczak, The University of Nevada, Reno / Natural Resources and Environmental Science

River and stream biodiversity and water quality are sensitive to anthropogenically altered chemical regimes. Salt inputs, like those released from irrigation return flows and road deicing salt applications, mobilize nutrients and metals from river sediments into the water column with consequences for river ecology, agriculture, and drinking water sources. The associated effects of temporally fluctuating salt inputs on stream and river biotic and abiotic factors are not well characterized. To understand how varying salt inputs affect these factors, we developed stream mesocosm experiments to manipulate salt concentration and input intervals. We will quantify associated changes in microbial biomass and extracellular enzyme activity, as well as surface water and sediment chemistry in streams draining watersheds with upstream urban, agricultural, and undeveloped land cover in northwestern Nevada. We anticipate that changes in water quality and microbial communities will be the most pronounced in constant elevated salinity treatments versus pulsed and low salinity treatments. Our results will help characterize variation in river and stream chemistry and biotic communities exposed to different salt regimes.

02.09.12 Biochar-Amended Engineered Streambeds for the Attenuation of Pesticides and Pharmaceuticals in Stormwater

A. Portmann, B. Halpin, C.P. Higgins, J.E. McCray, Colorado School of Mines / Civil and Environmental Engineering

Due to its unique properties, the hyporheic zone (HZ) has been called a natural bioreactor and the "River's Liver", which can attenuate various point and nonpoint source contaminants. Despite substantial water quality challenges, current stormwater management strategies typically prioritize water quantity issues over pollutant removal. To increase HZ exchange and ultimately improve water quality, we introduced a novel stormwater control measure (SCM) featuring modifications to streambed hydraulic conductivity and reactivity - called Biohydrochemical Enhancements for Streamwater Treatment (BEST). This research was conducted using an outdoor pilot-scale flume facility installed at the Colorado School of Mines in Golden, CO. The flume facility consisted of four individual streams (15 m length each) with the following configurations: 1) Sand streambed, 2) sand streambed with BEST structures, 3) sand streambed with BEST structures and biochar (7 vol%), and 4) fine sand streambed with BEST structures and biochar (7 vol%). The overall objective of the study was to assess the removal and potential transformation of select pesticides and pharmaceuticals in BEST engineered streambeds during a flow-through experiment simulating a 4-hour rain event. When biochar was added, the BEST streambeds led to removal of most tested organic contaminants in the surface water: For example, the influent concentration peak of atrazine, a well-known herbicide, was reduced by 64% along the 15 m stream reach. Furthermore, the data collected during these experiments will be used to estimate reach-scale contaminant attenuation rates and to predict removal under different streambed configurations and dimensions.These results show that BEST may be an effective novel SCM for improving stormwater quality with important practical implications: Reducing concentration peaks in stormwater effluents has aquatic life and human health benefits, especially when stormwater is released into surface waters or infiltrated into the ground.

02.09.13 Metals Retention in Biochar-Amended Engineered Streambeds for Stormwater Treatment

B. Halpin, A. Portmann, C.P. Higgins, J.E. McCray, Colorado School of Mines / Civil and Environmental Engineering

Stormwater runoff frequently contains elevated metals concentrations due to wash-off from roadways, buildings, industrial sites, and other impervious surfaces. When left untreated, stormwater can convey these metals to nearby surface waters, causing water quality impairments. To improve the quality of this runoff, a novel treatment technology, known as Biohydrochemical Enhancements for Streamwater Treatment (BEST), has been developed. Applying knowledge of contaminant attenuation processes that occur in the natural hyporheic zone, BEST is a streambed engineering approach for constructed drainage channels that incorporates: (1) hydraulic conductivity modifications along the streambed reach to increase surface-subsurface exchange, and (2) geomedia amendments in the streambed to promote reactions of interest. To better understand the fate of stormwater-relevant metals in BEST, we constructed four engineered streambeds in separate channels at an outdoor flume facility located at the Colorado School of Mines (Golden, CO). The following streambed configurations were studied: (A) an all-sand streambed, (B) a sand streambed with hydraulic conductivity modifications, (C) a sand and biochar (7vol%) streambed with hydraulic conductivity modifications, and (D) a replica of streambed C, but constructed with finer sand. To study the fate of metals, we introduced cadmium, copper, nickel, lead and zinc to the flumes' influent for 4 hours, simulating a storm event. At the downstream end of each 15m flume, the surface water peak concentrations of all five metals were reduced compared to what was measured upstream; this occurred in all four flumes. However, the addition of biochar in Flume C led to the greatest water quality improvements along the 15m reach, with the peak concentrations of all five metals reduced by more than 50%. Overall, these results indicate that engineered streambeds – particularly those constructed with biochar - have the potential to capture metals present in stormwater runoff. However, future work is required to investigate the potential for metals desorption, the service life of BEST, and disposal options for spent streambed media.

02.09.14 Greenhouse-Scale Comparison of Native Oregon Plants for Bioswale Remediation of Per- and Polyfluoroalkyl Substances (PFAS) in Stormwater Runoff

B. Parker, Oregon State University / Chemistry; R. Hilliard, T.S. Radniecki, Oregon State University / Chemical, Biological, and Environmental Engineering; S. Simonich, Oregon State University / Chemistry / Environmental and Molecular Toxicology; J.A. Field, Oregon State University / Environmental and Molecular Toxicology

Per- and polyfluoroalkyl substances (PFAS) are ubiquitous contaminants of concern that are often found in stormwater. Recent efforts to remediate stormwater focus on using green infrastructure best management practices (BMPs) because they are low cost, low maintenance, and effective at preventing recontamination of receiving sediments and waters. Such remediation strategies include bioswales, which tend to be vegetated channels. Shorter-chained PFAS are problematic to remove because of their high water solubility and mobility, but they are known to accumulate in plants. However, there are few studies utilizing plants as a remediation technique and little is known on how to optimize selection of plants to include in bioswales and other stormwater treatments for the most efficient removal of PFAS. A 10 week greenhouse experiment was conducted to quantify the removal and transformation of PFAS by 10 different plants native to Oregon, including multiple species of grasses, rushes, sedges, stemless dicots, and stemmed dicots. Each plant was watered with stormwater containing a mixture of perfluoroalkyl carboxylates, sulfonates, and sulfonamides with a variety of chain lengths. Aqueous, soil, and plant samples were extracted and analyzed using liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QToF/MS). At the end of 10 weeks plants were compared for bioconcentration factor (BCF), translocation factor (TF) and sulfonamide transformation. These factors were used determine which plants were the most effective at removing PFAS from stormwater. Some plants were much more effective at removing PFAS, particularly the shorter-chain compounds. In general, grasses and stemless dicots have significantly higher BCFs than other groups of plants. Additionally, stemless dicots have the highest TFs, indicating higher efficiency of transporting PFAS from root to shoot. Multiple plants showed a net production of perfluoroalkyl sulfonates relative to the no plant control, indicating the transformation of the C6 (FHxSA) and C8 (FOSA) perfluoroalkyl sulfonamides. Concurrent experiments with the same plants indicate their potential use for removal of other contaminants including heavy metals and polycyclic aromatic hydrocarbons (PAHs).

02.09.15 Impact of Bioretention Internal Water Storage (IWS) Underdrain Height on Nitrogen Removal

A.G. Donaghue, E. McKenzie, Temple University / Civil and Environmental Engineering

Owing to abundant impervious surfaces, urbanization increases total runoff volume and peak discharge. Additionally, pollutant loads, for a range of contaminants including nitrogen, can be high and negatively impact waterways. Green stormwater infrastructure (GSI), such as bioretention, targets water quantity and quality improvements via infiltration-based approaches and many contaminants can be effectively managed through sorption. However, nitrate often remains mobile and management requires the creation of an anaerobic internal water storage (IWS) to promote denitrification, with the intent of converting nitrate into nitrogen gas. This study used 2-D columns to evaluate how the underdrain height within the IWS impacts nitrogen removal. Steady state conditions confirmed that hydraulic residence time was a powerful predictor for nitrate removal and zeroth order kinetics were observed. IWS contained dissolved organic carbon and dissolved oxygen remained less than 0.2 mg/L indicating favorable denitrifying conditions. The presence of immobile zones below elevated underdrains (identified in previous tracer studies) did not notably contribute to nitrate removal. Transient experiments employed intermittent flow to mimic storm events and confirmed substantial nitrate removal occurred between storms. While "new" stormwater containing elevated-nitrate water pushed out "old" low-nitrate IWS water, the data suggested that a portion of nitrate removal occurred during the storm. Specifically, the bottom underdrain resulted in the lowest storm-event based N export, due to the increased IWS effective pore volume and hydraulic residence time. Over several months of experimental work, woodchip leaching of dissolved organic carbon decreased and may have contributed to reduced nitrate removal performance. A second phase of research currently underway includes dissolved organic carbon in the feed water. Previous field studies demonstrated that dissolved organic nitrogen and nitrate dominate infiltrating stormwater. Therefore, consideration of dissolved organic carbon and nitrogen fate is important for understanding bioretention IWS performance. Interestingly, the presence of dissolved organic carbon decreased the overall nitrogen and nitrate removal; on-going experiments will evaluate the fate of a range of nitrogen species under steady state and transient conditions.

02.09.16 Iron-Enhanced Sand Filtration (IESF) on Contaminant Mitigation and Source of Contamination Using Environmental DNA in Urban Stormwater

S. Kohno, St. Cloud State University / Biology; J. Gerads, H.L. Schoenfuss, St. Cloud State University / Aquatic Toxicology Laboratory

Urban stormwater contains a variety of pollutants, including contaminants of emerging concern. It is essential to reduce the level of pollutants before they are released into the environment. Our objectives are: (1) How the stormwater treatments in the regular pond and iron-enhanced sand filtration (IESF) mitigates biologically active contaminants in urban stormwater; (2) Where is the source of contaminations in urban stormwater. Both analytical chemistry and biological endpoints were utilized to investigate the mitigation potential of stagnant ponds and iron-enhanced sand filtration (IESF) *in vitro* and *in vivo*. Inflow and effluent samples were collected from seven stormwater ponds in St. Paul, Minnesota, USA, metropolitan area following precipitation events in different seasons in 2018-19. Fathead minnow larvae were tested for survival, consumption, and escape response after 21 days of developmental exposure to urban stormwater. In addition, an *in vitro* cell culture system was employed to analyze the biological activity of stormwater. Seasonality affected the concentration of Nonylphenol stormwater with a peak in spring, but no effect of treatment on nonylphenol concentrations. IESF-treatment reduced larval survival in exposures to stormwater collected in spring and summer, while larval consumption was affected by treatment in fall-collected stormwater exposures. At 24 h exposure to stormwater, none of the sites or treatments affected estrogenicity via fathead minnows estrogen receptor 1 *in vitro*, whereas the treatments affected cell viability among sites at 6 h exposure to stormwater *in vitro*. The presence of species-selective environmental DNA revealed multiple sources of contaminations to urban stormwater, including human sewage, dog waste, and feces from waterfowl. These results showed that the potential mitigation of reservoirs for stormwater treatment varied by season. Some contaminants detected in urban stormwater are likely the result of direct, untreated sewage inputs to stormwater from either human or pet waste.

02.09.17 Longevity of Bioretention Depths for Preventing Acute Toxicity from Urban Stormwater Runoff

L.W. Maguire, Washington State University / School of the Environment; J. McIntyre, Washington State University / Puyallup Research and Extension Center; J. Davis, U.S. Fish and Wildlife Service / Washington Fish and Wildlife Office

Urbanization poses increasing threats to aquatic ecosystems including increased chemical loading. The impacts of urbanization on biological integrity are especially evident in the lowland, urban streams of western North America, where adult coho salmon (*Oncorhynchus kisutch*) returning to spawn in the fall have been prematurely dying at high rates. Previous studies have demonstrated the effectiveness of bioretention systems in treating urban stormwater runoff and preventing acutely lethal and sublethal effects to aquatic organisms. The current study aims to determine the effectiveness and longevity of bioretention soil media over time at various infiltration depths, including those shallower than 18 inches, the depth currently required by the Washington Department of Ecology. Stormwater runoff was collected from a busy, urban road site and applied to experimental columns, containing five different depths of bioretention soil media. Experimental columns were dosed with runoff at an accelerated rate in order to simulate six water years in approximately 15 calendar months. The chemical and biological effectiveness of the columns in treating runoff was assessed using analytical chemistry and the health of juvenile coho salmon. Bioretention treatment efficiently removed copper, zinc, total PAHs, and total suspended solids (> 70% removal). Although all treatments continued to export nitrates after six accelerated years, the export of nutrients was greatly reduced by the end of the first accelerated year. Influent stormwater runoff was acutely lethal to juvenile coho salmon (>88% mortality in three exposures). However, treated effluent stormwater completely prevented coho morality for all bioretention depths and for all three exposures, indicating a continued ability to prevent acute lethal toxicity after six accelerated years of treatment. This study is ongoing and will continue to assess bioretention effectiveness through 10 accelerated years.

02.09.18 Phosphate Removal from Urban Stormwater Runoff by Biofilters Amended with Different Sorbents: Long-Term Column Studies Under Intermittent Flow Conditions

N. Esfandiar, Temple University / Civil and Environmental Engineering; R.P. Suri, Temple University / NSF WET Center; E. McKenzie, Temple University / Civil and Environmental Engineering

Urban stormwater runoff is a major source of potentially harmful pollutants to receiving water bodies such as lakes and rivers. Biofiltration systems are increasingly being used as a stormwater management practice in urban areas, with the intent of using infiltration to mitigate both stormwater quality and quantity challenges. While biofiltration soil media is efficient for the treatment of multiple pollutants, in many cases the media is ineffective in removing excessive stormwater nutrients such as phosphate that may cause surface water eutrophication; sorbent amendments may be required to enhance pollutant removal. In this study, a laboratory-based column study (30 cm media depth) was conducted to investigate the ability of three sorbent materials: coconut fiber (CF), blast furnace slag (BFS) and scrap tire (ST) as amendments to biofiltration media (sandy loam), for the removal of phosphate from simulated stormwater (SSW). Sorbent media were evaluated at 5% incorporation by volume with sandy loam as a control. The biofilters were dosed with SSW in an intermittent flow condition according to two scenarios: (1) four months moderate rainfall events with 2-3 antecedent dry days, (2) one month high-intensity rainfall events with one antecedent dry day. Results showed that among all filter media, BFS-amended media had the highest phosphate sorption capacity and adsorbed > 90% of the influent phosphate over 5 months experiments (moderate and high-intensity rainfall events). In non-amended control and CF-amended biofilters, effluent phosphate concentration increased to $C/C_0 \sim 40\%$ at the end of 4 months (moderate rainfall) and to $C/C_0 > 90\%$ at the end of one month high-intensity rainfall events (almost full exhaustion of media). ST-amended media reached

C/C0 ~ 20% at the end of 4 months and 70% at the end of 5 months. The phosphate removal efficiency of biofilters followed the order of BFS >> ST > CF ~ Control. Overall, with the phosphate influent concentration of 1000 µg/l, BFS-amended media consistently produced phosphate effluent mean concentrations < 10 µg/l and showed a maximum effluent concentration of 60 µg/l. High phosphate removal capacity of BFS is likely due to elevated calcium (Ca) content and alkaline conditions (pH~9) leading to phosphate removal mainly through Ca-Phosphate precipitation. The ST media contained elevated Zn and BFS media leached some Al into the treated water, which may pose a concern and require pretreatment prior to use.

02.09.19 Removal of Polycyclic Aromatic Hydrocarbons and Fecal Indicator Bacteria from Stormwater Using Amended Bioretention Soils

C.J. Mitchell, A. Jayakaran, J. McIntyre, Washington State University / Puyallup Research and Extension Center

We evaluated the treatment of Polycyclic Aromatic Hydrocarbons (PAHs) and Fecal Indicator Bacteria (FIB) in highway runoff by bioretention. We compared treatment performance by Washington's standard bioretention mix of 60% sand, 40% compost (by volume), and by three other blends amended with biochar and fungi. Biochar-amended mixtures contained 60% sand, 20% compost, and 20% biochar. Fungi-amended mixtures were inoculated with *Stropharia rugosoannulata*. Twelve bioretention columns were constructed in a greenhouse, each containing 18" of bioretention mix planted with *Carex oshimensis* overlaying a 20" gravel drainage layer. Columns were initially conditioned with clean water and then dosed with stormwater collected from a highway downspout in Tacoma, WA during 8 events over a 14-month period. Effluents for each column were analyzed for 23 PAHs, *E. coli,* Fecal coliform, dissolved organic carbon (DOC), and total suspended solids (TSS). To track the fate and transport of PAHs within the bioretention columns, soil cores were taken four time throughout the study. TPAHs were almost completely removed by all treatments across all storms, with removal rates ranging from 97-100% for 94 out of 96 samples. Compost appears to be a source of PAHs in bioretention soils, as biochar-amended soils initially contained half the soil TPAHs as columns with the standard 60:40 sand:compost mixture. We observed a net loss of TPAHs between ranging 19-73% in bioretention soils which could not be explained by PAHs in effluent alone, suggesting that microbial bioremediation and/or plant uptake attenuated soil PAHs. Fungi amended bioretention soil showed higher TPAH losses (70-73%) than bioretention soil alone (60-71%). Influent concentrations of FIB spanned 3 orders of magnitude. *E. coli* and fecal coliform were initially exported, but all columns achieved some treatment after the first dosing event.DOC was exported from all columns, but export was reduced in biochar-amended columns.

02.09.20 Effectiveness of Bioretention Systems to Reduce Microplastic Pollution in Portland, Oregon

J. Wolfand, C. Poor, University of Portland / Civil Engineering; B. Taylor, University of Portland / Chemistry

Stormwater is a potential major pathway of microplastic pollution to fresh and coastal water bodies. Green infrastructure such as green roofs, bioretention cells, and bioswales, are increasingly installed to reduce flood risk and remove conventional and emerging stormwater contaminants. In this study, we investigate baseline concentrations of microplastics in stormwater in Portland, OR and test the removal efficiency of microplastics in bioretention columns. Microplastics were quantified in stormwater grab samples taken at five catch basins over nine storm events using visual identification and Fourier-transform infrared spectroscopy (FTIR). Laboratory scale bioretention columns packed with three geomedia (conventional sand-compost mix, proprietary sand-compost blend, and layered compost/sand) were dosed with collected stormwater and synthetic microplastics. A total of 5 tests were conducted to evaluate the efficacy of microplastics removal in bioretention cells. Results will inform future microplastics monitoring and green infrastructure installation efforts in Portland.

02.09.21 Green Infrastructure on Vacant Land: Mitigating Aquatic Stressors of Urban Ecosystems Through Green Stormwater Infrastructure

G.A. Burton, University of Michigan / School for Environment and Sustainability; M. Hudson, EA Engineering, Science, and Technology, Inc., PBC / Water and Natural Resources; S. McElmurry, Wayne State University / Civil & Environmental Engineering; C. Riseng, J. Iverson Nassauer, The University of Michigan / School for Environment and Sustainability

Green Stormwater Infrastructure (GSI) is becoming a widespread stormwater management practice. GSI aims to reduce flooding and combined sewer overflows, with the ultimate goal to improve water quality. The city of Detroit, Michigan has experienced a high amount of vacant property demolition throughout the vast city limits. This research aimed to understand the usefulness of demolishing vacant properties, filling in the basement with approved materials, then draining street runoff through a planted basin then the "basement" filtration, prior to emptying into storm drains. Surface and groundwater flows were monitored along with water quality and toxicity. *Daphnia magna* survival and reproduction in "first flush" exposures following the on-site natural filtration. Statistically different ($p < 0.00001$) survival was noted at the inlet versus outlet across both locations. High nutrients, metals and PAHs were reduced along with surface water loadings. Surface water volume was reduced an average of 86% in rainfall events smaller than the 10-yr event. Small increases in nutrients and TDS at the outlets were insignificant, as mass loadings were dramatically reduced. Some of the increases likely resulted from groundwater intrusions into the GSI systems. These results suggest that, with proper maintenance, these GSI systems significantly reduce stormwater volume and increase stormwater runoff quality.

02.09.22 The Relative Impact of Urban Watersheds on Estuarine Sediment Quality

K. Schiff, K. McLaughlin, Southern California Coastal Water Research Project

Urban watersheds are a known source of many potential pollutants including nutrients, trace metals and pesticides. The potential for environmental impacts at the bottom of urbanized watersheds is particularly pronounced in Southern California, where urbanization along its 350 km coastline is especially dense and whose estuaries are typically small and modified with limited circulation. Compounding this problem are the region's infrequent but intense rainfall events, combined with separate sanitary and storm drain systems, so urban runoff receives little to no treatment prior to discharge. This presentation examines the areal extent and magnitude of sediment quality impacts in Southern California, compares these impacts among the different types of estuaries, and then correlates these impacts with watershed characteristics. The data is from a long-term probability-based regional study that sampled every estuary from Santa Barbara to San Diego measuring sediment chemistry, sediment toxicity, and infaunal biological communities. These sediment quality results were compared to a variety of watershed characteristics including land use, size, and estuary development. Ultimately, an estimated 50% of the sub-tidal soft-bottom area in estuaries of Southern California exceeded State regulatory sediment quality objectives (SQOs), an areal extent that has decreased since monitoring began in 2003, but still remains the greatest areal extent and magnitude of SQO exceedances of any of the 11 near-costal habitats in Southern California. Watershed characteristics had a significant relationship to sediment quality, in particular sediment chemistry, with the most important factors being relative proportion of industrial and open land uses. However, estuary configuration had an equally or greater contribution to the relationship with sediment quality. More modified estuaries had greater sediment quality impacts compared to less modified estuaries. This information is being used by local environmental managers for stormwater permitting, 303d listing and TMDL implementation, and restoration planning.

02.09.23 The Revised MSGP and Tools Related to the Development of Site-Specific Copper Benchmark Values

K.J. Rader, T.L. Torralba-Sanchez, Mutch Associates, LLC; R.F. Carbonaro, Manhattan College / Chemical Engineering; E. Smith, C.A. Claytor, Copper Development Association Inc.

On January 15, 2021, the U.S. Environmental Protection Agency (EPA) issued a revised version of the Multi-Sector General Permit (MSGP) for stormwater discharges related to industrial activity. The MSGP is applicable in Idaho, Massachusetts, New Hampshire, and New Mexico, as well as all Indian country lands and federal operators in selected states. It, however, also serves as a model for many state permits. The 2021 MSGP requires facilities to, among other things, conduct benchmark monitoring of several water chemistry parameters and develop site-specific stormwater pollution prevention plans (SWPPPs). One of the modifications introduced is a new freshwater benchmark threshold for copper (Cu) changed to 5.19 µg/L from a hardness-based range in the 2015 MSGP. The new benchmark was derived using the Copper Biotic Ligand Model (BLM). This model quantifies the form copper takes based on ambient water chemistry and the associated copper bioavailability (i.e., its ability to cause toxicity). The freshwater copper benchmark of 5.19 µg/L was derived with the BLM and a national dataset of receiving water chemistry parameters including pH and dissolved organic carbon (DOC). For the 2021 MSGP, EPA allows facilities that repeatedly exceed certain benchmarks, including Cu, to discontinue comparisons to national benchmarks and instead demonstrate that their discharge(s) would not result in an exceedance of a derived site-specific value. For Cu, the site-specific value is calculated using the BLM and a suite of model input water quality parameters that must be measured a prescribed number of times at locations upstream (if applicable) and downstream of the discharge. Facility managers must decide if the development of a site-specific Cu benchmark is worthwhile, or if the resources required to do so would be better spent in other ways to address benchmark exceedances. A simple screening approach was developed to inform facility managers whether the national benchmark is potentially overprotective based on a limited number of site-specific receiving water parameters. An analysis was conducted to assess the reliability of the screening tool. Areas for additional decision-support tool development are discussed.

02.09.24 Persistent and Mobile Organic Compound Fate in a Bioretention Cell Predicted Using the Novel Bioretention Blues Model and a Spike and Recovery Test

T.F. Rodgers, University of Toronto / Chemical Engineering and Applied Chemistry; L. Wu, University of Toronto / Departments of Civil & Mineral Engineering; X. Gu, University of Toronto / Chemical Engineering and Applied Chemistry; S. Spraakman, University of Toronto / Department of Civil and Mineral Engineering; E. Passeport, University of Toronto / Department of Civil and Mineral Engineering and Department of Chemical Engineering and Applied Chemistry; M.L. Diamond, University of Toronto / Department of Earth Sciences

The large concentration of people in urban areas causes cities to act as sources for many pollutants to the surrounding region in a process which has been called the “Urban Halo” effect. Stormwater and wastewater treatment plants concentrate numerous small point source and diffuse emissions, as they convey the pollutants to the environment. Stormwater is an important source of semi-volatile organic compounds (SVOCs) as it washes off the “urban film” on impervious surfaces, upon which many hydrophobic SVOCs accumulate during dry periods and which can mobilize hydrophilic persistent and mobile organic compounds (PMOCs). Benzotriazole, with many uses including as a corrosion inhibitor in products like aircraft deicing fluids, and some organophosphate esters (OPEs), used as plasticizers and flame retardants, are examples of PMOCs that have been found in stormwater runoff. Bioretention cells are an emerging stormwater management technology that are intended both to reduce the quantity of water entering receiving bodies and to improve the receiving water’s quality by removing contaminants. We developed a 1D multimedia model of a stormwater bioretention cell called “Bioretention Blues” and applied it to a spike and recovery experiment conducted on a system near Toronto, Canada, to investigate the fate of PMOCs, using benzotriazole and four OPEs for illustration. We found that most of the compounds with organic carbon partition coefficients (Log K_{OC}) 2.25 were mostly retained on the bioretention media where their fate depended on their transformation through biotic or abiotic processes in the media, with volatilization from the surface of the bioretention media predicted to play a significant role for volatile hydrophobic compounds with air-water partition coefficients (K_{AW}) >~-2 (e.g. 16% volatilization for simulated naphthalene, K_{AW} = -1.73) but a minor role for the compounds tested. Direct uptake by vegetation played a negligible role regardless of the compounds’ physicochemical properties, although the indirect impacts of vegetation on the system’s water balance impacted the ability of the system to retain more hydrophilic compounds.

02.10 Life Stage-Specific and Multi-Generational Effects of Early Life Exposure to Environmental Contaminants in Fish

02.10.01 Impact of Early Embryonic Exposure to PCBs & Aroclors Disrupt Development of Multiple Organ Systems

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Polychlorinated biphenyls, PCBs, were used in industry as mechanical lubricants and insulators, and as additives in pesticides and paints for decades due to their high stability under stresses. PCBs have become a significant environmental hazard as they were disposed of in local waterways and soils. PCBs bioaccumulate in fatty tissues, often causing developmental delays, reproductive problems and cancer. General Electric dumped 1.3 million pounds of PCBs into the Hudson River. These PCBs can now be found in the sediments around New York City and have made their way into people. PCBs are a classified by chemical structure as either coplanar or non-coplanar. These two main classes of PCBs are thought to differentially effect biological processes; typically, coplanar PCBs have a high binding affinity to aryl hydrocarbon receptor (AhR), while some non-coplanar PCBs have been shown to act as a ligand for estrogen receptor (ER). There are many studies exploring various aspects of TCDD and PCB exposure on model and wild organisms. However, few studies have compared PCB mixtures contaminating ecosystems to individual PCBs in the lab. The aim of this work is to addresses impacts of individual PCBs with PCB mixtures, Aroclors, in embryonic zebrafish to compare contaminant-derived defects in a lab model to a wild species living in contaminated waterways. We exposed zebrafish to PCBs and Aroclor mixtures at environmental levels and then assess the embryos at various life stages from day 3- 5 post fertilization for abnormal gross morphological changes in developing embryos and disrupted organ development. Cardiac development is retarded; specifically, we see failure of cardiac looping and chamber ballooning. In addition, gastrointestinal development is disrupted, the pancreas contains excess insulin positive cells and the liver is smaller. Utilizing Tg(5xERE:egfp) fish (ER pathway activation) and CYP1A immunohistochemistry (AhR pathway activation) we decipher the biological pathways affected by PCB and Aroclor exposure. Concentration dependent impacts on embryonic survival to adulthood was also assessed demonstrating a critical life stage at 5, 15 and 45 days post fertilization. Fish that survived past 45 survive a normal life span. This study demonstrates the value of using zebrafish as a model for the impact of aquatic pollutants on development and life span.

02.10.02 Non-Alcoholic Fatty Liver Disease in Medaka Caused by Ancestral BPA Exposure

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Bisphenol A (BPA) is a component of mass-produced plastics found in everything from plastic bottles to the coating inside metal cans. It has been linked to a multitude of adverse endocrine-disrupting health effects, as it can interact with estrogen, androgen, and thyroid hormone receptors. It has also been linked to the modification of genes that affect lipid synthesis and accumulation, including de novo fatty acid synthesis. Accumulating evidence suggests a role of BPA in the development of adverse liver health outcomes, including non-alcoholic fatty liver disease (NAFLD). NAFLD is a condition of excess fat accumulation in the liver of animals and humans. Moreover, all observed adverse liver health outcomes are based on only the direct exposure of animals and humans to BPA. Thus, there is a research gap in determining whether grandparental BPA exposure can lead to NAFLD in grandchildren who are not exposed to it. To address this question, the livers of the medaka (*Oryzias latipes*) fish whose grandparents were exposed to an environmentally relevant concentration of BPA (10 ug/L) during their first 12 days of embryonic life were histologically examined along with paternal sperm epigenome and grandchildren's liver transcriptome. Results suggest that BPA induces heritable differential DNA methylation profile (epimutations) in the primordial germ cells that can survive reprogramming in the offspring somatic cells. Some of the epimutations that survive epigenetic reprogramming of primordial germ cells can be transmitted to a grandchild's body via germline transmission at F1 parental generation. Life transcriptome analysis shows a clear link between BPA-specific epimutations and differentially expressed genes in the liver, suggesting that there is a clear passage of germline epimutations to the liver cells of the grandchildren, leading to NAFLD. The NAFLD phenotype can be further transmitted up to 4 generations with a pronounced severity in females compared to males. The present findings suggest that BPA exposure can leave deleterious epigenetic memories in the germline which can be inherited by subsequent generations and may lead to severe liver pathologies. Such transgenerational adverse health outcomes may be threatening to fish at a population level.

02.10.03 Does Age Really Matter? Examining Age-Specific Behavior and Proteomic Responses of Zebrafish (Danio rerio) to a Model Toxicant

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Toxicological safety data for compounds used in both industrial and domestic settings is necessary to characterize and mitigate risk to both human and ecological health, yet is deficient for 86% of existing chemicals. These deficiencies, coupled with the significant time and monetary costs of traditional toxicity testing, have prompted a shift away from historically used methods towards those that reduce the number of animals, leverage existing data, and implement high throughput screening (HTS) techniques. Due to the high fecundity, rapid development and sensitivity, current in vivo screening measures commonly utilize embryonic fish as toxicity models. Zebrafish (Danio rerio) in particular share a high degree of genetic similarity with humans (over 70% of human genes have a zebrafish ortholog), making them a popular model for both human health and toxicological studies including the fish embryo toxicity (FET) test. While the FET test typically exhibits a high level of correlation with the traditional Acute Fish Toxicity test (AFT), there is evidence that FET demonstrates weaker toxicity than AFT for some compounds. Previous research in our lab has shown that larval fish exposed to the antihistamine diphenhydramine (DPH) demonstrate increased mortality, uptake, and behavioral toxicity than their embryonic counterparts. However, whether dispositional and/or molecular initiation events influenced such age-specific differences were not identified. The current research targets this literature gap by examining changes in proteome expression of zebrafish exposed to DPH at sublethal concentrations across embryonic, larval, juvenile, and adult life stages. Over 3200 proteins were identified (1 % false discovery rate) and grouped based on gene ontology resulting in differential expression of proteins implicated in morphogenesis, angiogenesis, and neural development. In comparisons of age specific functional differences, genes associated with chemoattraction, neuron generation, and ion gated channel activity showed differences in expression across timepoints. By contrasting changes in whole-body protein expression with chemical exposure across age groups, this study identified age and exposure-specific differences in the identity, proportion, and function of identified proteins.

02.10.05 Endocrine Disruption at Single-Cell Resolution in the Dichlorodiphenyltrichloroethane (DDT)-Exposed Medaka Ovary

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Although concentrations have declined globally over the past few decades, the highly persistent dichlorodiphenyltrichloroethane (DDT) and related chemicals are a persistent problem. The recent discovery of >27,000 barrels of DDT production waste offshore of Southern California has called the long-term ecological and human health impacts of legacy contamination into question. Epidemiological studies have revealed associations between in utero exposure to DDT and increased risk of female reproductive cancers and infertility. However, experimental studies have yet to demonstrate causal mechanisms and it is unclear how early developmental chemical exposures contribute to adult onset of disease. This project aims to better understand ovarian endocrine disruption induced by early life xenoestrogen exposure. We will pair the Japanese medaka (Oryzias latipes) teleost fish model with cutting-edge single-cell transcriptomics (scRNA-seq) to test the hypothesis that early life stage exposures to estrogenic o,p'-DDT induces transcriptional effects in ovarian somatic gonad cells, reducing fertility later in life. The medaka model has an extensively mapped genome, embryonic sexual dimorphism, and short generation time enabling sex-specific exposures at various developmental windows and timely evaluation of adult disease. Moreover, it's chromosomal sex determination and shared estrogen receptor conformation with humans make it a useful vertebrate model for human disease research. Female medaka larvae will be exposed for 30 days to physiologically relevant o,p'-DDT levels during a key window of ovarian differentiation, then grown out to sexual maturity. Functional reproductive outcomes resulting from early life exposure will be evaluated via breeding assessments and ovarian histopathology. To further characterize heterogeneous transcriptional responses of specific ovarian somatic cell types, scRNA-seq will be performed on developmentally o,p'-DDT-exposed ovaries and compared to controls. Identifying cellular responses at single-cell resolution in the xenoestrogen-exposed ovary may identify novel estrogen-responsive target genes, as well as downstream signaling pathways that may contribute to reproductive disease. Furthermore, the proposed work will develop and evaluate the application of comprehensive single-cell transcriptomics to the medaka in vivo model with the long-term goal of enhancing predictive toxicology screening of endocrine disruptors at the cellular level.

02.10.06 Maternal and Paternal microRNAs Play a Role in Generational Responses to Mixed Stressors in Zebrafish

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Increased urbanization and climate change-related stressors pose a serious threat to aquatic species. These stressors present complex mixtures of multiple organic and abiotic stressors that can affect fish both intra- and intergenerationally. Evidence suggests that there is a strong maternal influence on the phenotypic response of offspring to multiple stressors, but the direct contribution of either maternal or paternal influences isn't as clear. Here we present evidence for the generational influence of multiple stressors in zebrafish, examining both transcriptional and microRNA expression following exposure to increased temperature, hypoxia, and venlafaxine (VEN), an antidepressant and known surface water contaminant. Furthermore, we have demonstrated that there is a significant response in microRNA abundance in the gonads of both male and female zebrafish following a combined exposure to VEN and increased temperature, suggesting that there may be separate maternal and paternal influences on embryo development. Building upon these results, we used mating pairs of zebrafish to examine the microRNA contribution from either maternal or paternal sources. Zebrafish were bred and then either the male or female from the breeding pair was exposed to either hypoxia or VEN for 2 weeks. Following exposure, the mating pair was bred again, and embryos were collected immediately following fertilization. Results indicated that there are significant differences in the microRNA abundance in the embryo following exposure, and evidence suggests that there are differential contributions from maternally and paternally exposed zebrafish. Combined, this research demonstrates the impact of realistic, mixed stressor exposures on early life development and identifies significant differences when it comes to maternal and paternal epigenetic influences on developing embryos

02.10.07 Later Life Bioenergetic Consequences of Embryonic PAH Exposure and Increased Temperature in Subpopulations of Atlantic Killifish From the Elizabeth River

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The Elizabeth River (ER) is an estuary in Virginia that experienced decades of creosote pollution, resulting in sediments with significant polycyclic aromatic hydrocarbon (PAH) contamination. Creosote pollution was largely caused by three major wood treatment facilities that operated on the river for most of the 1900s: Atlantic Wood Industries (AW), Republic Creosoting (REP), and Eppinger and Russell (Money Point; MP). These sites, along with the moderately contaminated Jones Creek (JC), a small tributary of the ER about 1 km upriver of the AW site, and the relatively uncontaminated King's Creek (KC), a tributary of the Severn River, represent a gradient of PAH contamination. Atlantic killifish (*Fundulus heteroclitus*) that reside in the ER have evolved resistance to PAHs, though this PAH-adapted phenotype has been associated with fitness costs, including increased susceptibility to disease and lower tolerance for hypoxia. Furthermore, adult killifish from the ER exhibit organismal-level bioenergetic shifts, including decreased aerobic scope and reduced thermal tolerance. While developmental effects of embryonic exposure to PAHs are relatively well-established, less is understood about the later life consequences of early life exposure to PAHs in killifish, including impacts on mitochondrial health. It is also unknown how increased temperature affects the development of these PAH-adapted offspring. Here, we exposed embryos collected from adults from KC, JC, MP, AW, and REP sites to 0 or 1% ER sediment extract (ERSE) during 24-144 hours post fertilization and then raised to the juvenile stage. Experiments were run in parallel at 28 and 32 °C. Juvenile killifish were tested for whole organismal metabolic rates using a swim tunnel respirometer and heart and brain-specific mitochondrial function were measured using the Seahorse XF24 Extracellular Flux Analyzer. Juveniles from ER populations had significantly lower metabolic rates than KC and were not impacted by early life ERSE exposure, suggesting that the adapted phenotype of ER is protective against later life bioenergetic consequences of early life PAH exposure, but with an altered metabolic profile. Juvenile respirometry data from subpopulations with a gradient of PAH pollution serve as indicators of fitness and provide important risk assessment data for Atlantic killifish, an integral species in the ER ecosystem.

02.10.08 Determining Epigenetic and Aryl Hydrocarbon Receptor-Mediated Impacts of Dietary Benzo[a]pyrene Exposure

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Polycyclic aromatic hydrocarbons (PAH), including benzo[a]pyrene (BaP), are implicated in many adverse outcomes in offspring of exposed parents such as: neural tube defects, growth deficits, cardiovascular defects, endocrine disruption, childhood cancers and infertility. However, the molecular mechanisms for the developmental and multi/transgenerational effects associated with PAH exposures have yet to be fully elucidated. The objectives of this study were to determine the role of the aryl hydrocarbon receptor (AHR) in the transcriptomic and epigenetic changes associated with preconceptional exposure to BaP. Adult 5D and AHR2-null (*ahr2osu1*) zebrafish were fed 708 µg BaP/g diet (measured) at a rate of 1% body weight twice/day (14 µg BaP/g fish/day) for 21 days. At the end of the 21-day parental exposure, fish were spawned using a crossover design, and behavioral effects were measured in 96 hpf F1 & F2, and adult F0 & F1. In the F0 5D line, there was no significant effect of BaP exposure on adult behavior, but in the F1 adults following BaP parental exposure, locomotor behavior was significantly affected in females. Larval behavior (96 hpf) assessed (light:dark assay) was significantly altered in both the F1 and F2 generations of 5D fish exposed to BaP. Transcriptomic effects and DNA methylation changes were measured using RNAseq and RRBS, respectively, on F0 sperm and eggs and the 10 hpf embryos from all 4 crosses. Differential methylation and transcriptomic changes were observed in all tissues tested. The embryos from crosses resulting from BaP males and control females had the largest number of differentially methylated regions and differentially expressed genes. In BaP-exposed *ahr2osu1* F0 fish, the male but not female fish displayed significantly reduced open-field behavior. However, the *ahr2osu1* F0 fish did not produce eggs in any treatment or control group, so effects on F1 fish were unable to be determined. These results demonstrate that dietary BaP exposure causes adverse developmental outcomes in multiple generations and suggest that males may contribute more to the multigenerational effect of BaP. Research supported by NIEHS 1R21ES0301.

02.10.09 Effects of Perchlorate Exposure on Germ Cells of Medaka Fish

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Perchlorate is a manufactured 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. Higher concentrations of perchlorate have been found to affect the reproductive system and whether lower environmentally realistic concentrations affect stem cells that produce sperm and eggs, also called primordial germ cells (PGCs), is not clearly understood. In the present study, I examined the effects of 0, 10, 100, and 1000 ug/L potassium perchlorate exposure on PGCs of medaka embryos and the expression pattern of the genes that maintain PGC integrity and epigenetic processes. Expression of gene encoding germ cells nuclear antigen (*gcna*) and DNA methyltransferases (*dnmt*) was determined by quantitative polymerase chain reaction (qPCR). Perchlorate exposure delayed hatching time, reduced heartbeat, inhibited migration of PGCs, increased developmental deformities, and reduced growth of embryos. The highest perchlorate concentration was lethal to embryos, whereas co-treatment of embryos with vitamin C (1 mg/L) completely rescued them from death. Expression of genes encoding proteins involved in catalysis of DNA methylation process (*dnmt3aa* and *dnmt3bb*) increased in PGCs of the 10 ug/L groups. In contrast, the expression of *gcna* showed a dose-dependent increase, suggesting that genomic integrity is being targeted by perchlorate. Gene Ontology enrichment analysis indicates that the proteolysis and metabolic process related pathway within the biological process category while peptides activity, hydrolase activity, and hormone activity in molecular function and extracellular, intracellular, sarcoplasmic, and 6-phosphofructokinase and membrane-bounded pathways in the cellular component category, was overrepresented from perchlorate. The results suggest that perchlorate affects germ cell nuclear integrity and epigenetic modulators in PGCs and provide insights into possible perchlorate effects on germline stem cells in humans. The results also provide insights into the role of vitamin C in mitigating the possible negative effects of perchlorate exposure on embryos.

02.10.11 Adverse Outcome Pathways Using Japanese Medaka Embryos (Oryzias latipes) Exposed to 2,3,7,8-Tetrachlorodibenzodioxin

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Adverse outcome pathways (AOPs) are an open, collaborative framework that categorizes the impact of chemicals from the molecular to the ecosystem level. This research aims to refine two existing AOPs that are initiated by dioxins and dioxin-like chemicals binding to the Aryl hydrocarbon receptor (AhR), ultimately resulting in an adverse outcome of altered cardiovascular development. Japanese medaka (Oryzias Latipes) hatch in nine days and their cardiovascular system can be observed using a Leica EZ4 stereomicroscope with ease daily due to their transparent nature. Embryos mature to morula stage only four hours post fertilization (hpf). At two days post fertilization (dpf) blood circulation begins distinctly with the pumping of the blood island and at seven dpf cardiac development is complete marked by the development of the pericardial sac around the heart. Our preliminary studies show that medaka (Oryzias Latipes) embryos exposed for 1 hour to 0.001 ppb of dioxin at 4hpf display altered cardiovascular development seen as reduced blood flow, pericardial edema, and impaired angiogenesis. When embryonic development is adversely impacted by dioxins, we hypothesize that there are differences in gene and protein expression, which will distinguish the molecular level key events in these AOPs. Medaka embryos were exposed to 0.001, 0.01, 0.1, 1, and 10 ppb of 2,3,7,8-Tetrachlorodibenzodioxin (TCDD) for 1 hour at 4hpf. Embryos were collected at 2 dpf and 7dpf for non-targeted proteomics and qPCR of targeted genes. Cardiac impairment was detected by heart rate analysis using open-source *HeartBeat* software on 7dpf videos and hatch malformation analysis was conducted by scoring of severity of pericardial edema. Targeted qPCR suggests activation of one of the two prospective AOPs while TCDD appears to be directly impairing hatching time and heart rate in Japanese medaka caused by early life stage exposure of the dioxin. Refining these AOPs will benefit society by improving our ability to respond to chemical contaminants of concern more effectively.

02.10.12 Assessing Copper Toxicity to Sockeye Salmon Fry Under Water Quality Conditions Analgous to the Freshwaters of the Bristol Bay Watershed

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Potential mining activities within the Bristol Bay watershed (BBW) have raised concerns over the possible impacts that releases of copper and other elements may have on the most valuable salmon fishery in the world. The bioavailability and toxicity of copper to fish is partially dependent on local water quality. For example, copper toxicity decreases with increasing water hardness and dissolved organic matter (DOM). Salmon populations within the BBW could be particularly vulnerable to copper toxicity due to the notably low hardness and DOM values found within the rivers in the region. The United States Environmental Protection Agency endorses the use of the Biotic Ligand Model (BLM) to account for water quality conditions and predict the bioavailability and lethal concentration values of copper to aquatic life. However, previous research has demonstrated that the BLM under-predicts copper toxicity for waters with low hardness and DOM. Accordingly, our research aims to assess the acute and sublethal toxicity of copper to sockeye salmon under water quality conditions relevant to the BBW. We are exposing sockeye salmon fry (*Oncorhynchus nerka*) to copper in a 96-hour flow-through bioassay with survival as an endpoint. The bioassay uses field-relevant water spiked with different concentrations of copper delivered via a flow-through proportional diluter. Additionally, we will expose sockeye salmon fry to sublethal concentrations of copper to evaluate olfactory inhibition. Olfactory inhibition will be evaluated by delivering field-relevant control water and water spiked with sublethal concentrations of copper. The test will be carried out as a flow-through, 24- to 96-hour test with periodic exposures to an olfactory response cue. Behavior will be quantified and analyzed using existing behavioral analysis software to determine if fish respond to the cue (no olfactory inhibition) or not (olfactory inhibition). By performing these bioassays using relevant water conditions, we will improve upon current methods for assessing the effects of copper, thus providing multiple stakeholders with the information needed to make informed decisions about the potential environmental impacts of local resource extraction.

02.10.14 Maternally Deposited 1,2,5,6-Tetrabromocyclooctane (TBCO) Impacts Reproduction of Japanese Medaka (Oryzias latipes)

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Brominated flame retardants (BFRs) are added to a variety of flammable products to increase their fire resistance. BFRs can leach from materials into aquatic ecosystems where they can bioaccumulate, biomagnify and induce toxicity in organisms. 1,2,5,6-tetrabromocyclooctane (TBCO) is an emerging BFR that is a potential replacement for the widely-used BFR, hexabromocyclododecane (HBCD). Little is currently known about the effects of TBCO on aquatic organisms. In a previous study, exposure of zebrafish (*Danio rerio*) embryos to waterborne TBCO caused developmental toxicity. In another study, dietary exposure of sexually mature adults to TBCO impaired reproductive performance of Japanese medaka (*Oryzias latipes*). During fish development, embryos can be exposed to the same effective internal concentration as the maternal organisms from which the eggs originated. Effects of maternally deposited TBCO on fish development and reproduction have yet to be studied. The goal of this study was to determine the effects of maternally deposited TBCO on development and reproduction of Japanese Medaka. Sexually mature fish (F0 generation) were fed either a control, low (100 µg/g), or high (1000 µg/g) dose of TBCO spiked fish food for 21 days. During the exposure, embryos were collected to assess maternal transfer of TBCO, developmental toxicity caused by exposure to maternally deposited TBCO, and for grow out to determine reproductive performance at sexual maturity. Concentrations of TBCO in eggs (F1 generation) were determined to be an average of 711.3 ng/g wet weight (ww) for low samples, and 2535.5 ng/g ww for high samples while concentrations in eggs of fish given the control diet were less than the method detection limit. In the F1 generation, embryos showed concentration dependent trends of increased functional mortality, increased incidence of swim bladder malformation, increased incidence of spinal curvature, and decreased heart rate. These results suggest that maternally deposited TBCO has similar effects as waterborne TBCO on early life-stages. Additionally, TBCO significantly impaired reproductive performance of the F1 generation. Cumulative fecundity of the F1 generation from F0 fish given the low and high TBCO diets were reduced by 44.6% and 42.7%, respectively, compared to the control. Effects of TBCO on reproduction of the F2 generation and molecular mechanisms of effects, including epigenetic mechanisms, are currently being investigated.

02.10.15 The Early Developmental Effects of BPA and BPA Analogues on Lipid Metabolism in Zebrafish

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To combat the global health challenge of obesity, industrial practices have transitioned from the utilization of bisphenol A (BPA), a known obesogen, to other bisphenol structural analogues (BSA) as a safer alternative. Due to increased usage, BSA are now detectable in surface waters; however, little is known about the metabolic consequences of exposures to these compounds in regard to the development of obesity. While structurally BSA exhibit similarities to BPA, our understanding of the toxicity of these compounds relative to BPA is still in its infancy. In this study we tested the hypothesis that BSA exhibits obesogenic effects similar to that observed for BPA. To investigate this, we first developed a model of increased lipogenesis in zebrafish by feeding them a ration of either 5, 25, or 50% total body mass from 6 to 15 days post fertilization (DPF). Excess feeding significantly increased body mass and adipose tissue deposition in the 15 DPF larvae. Coinciding with this increased adipogenesis were the upregulation of transcript abundance of several key markers of lipogenesis, including fatty acid synthase, elongation of very long chain fatty acid protein 2, and diacylglycerol O-Acytltransferase 2 in the excess fed larvae. Using the 25% fed group as our positive control group for the obesogenic phenotype, we compared lipid accumulation in response to early developmental exposures to BPA and BSA at waterborne concentrations of 1, 10, and 100 µg/L from 2 hours post-fertilization to 15 DPF. Zebrafish were then assessed for changes in key molecular markers of adipogenesis along with total fat and triglyceride contents. The results from this study will demonstrate whether early developmental exposure to BSA increases adipogenesis, leading to an obese phenotype in zebrafish.

02.10.16 Metformin Exposure Impacts Development of Wild-Spawned Embryo-Larval Fishes

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The occurrence and fate of pharmaceuticals in the aquatic environment is a rapidly evolving issue of considerable importance within the field of aquatic toxicology. This is particularly true for highly prescribed pharmaceuticals present in wastewater treatment plant (WWTP) effluents, which often exist in a state of pseudo-persistence within receiving waters. As one of the most widely prescribed pharmaceuticals, metformin (prescribed for the treatment of Type 2 diabetes) and its metabolically active transformation product, guanylurea, are routinely detected in surface waters globally. Laboratory studies have shown that developmental exposure to environmentally relevant concentrations of metformin/guanylurea leads to changes in key metabolic pathways and adversely impacts the growth of early life stage (ELS) fish; however, there is a paucity of data describing the potential effects of exposure on wild fish populations. To address this data gap, wild-spawned fathead minnow (Pimephales promelas) embryos were collected from a natural boreal lake at the IISD-Experimental Lakes Area in Northwestern Ontario, Canada and subsequently exposed to 0, 4, or 40 µg/L metformin through hatch (7 days post fertilization [DPF]). Exposure water was sub-sampled from in-lake mesocosms spiked with metformin, to account for the effects of natural processes on the bioavailability and potency of metformin to exposed aquatic organisms. Newly hatched larvae were imaged and analyzed for a suite of morphological parameters using ImageJ. Study results revealed changes in several developmental endpoints in all exposed animals (regardless of treatment) that correspond with the findings of laboratory studies, including dysregulation of important metabolic processes and morphogenesis. Additionally, significant adverse effects on eye development were observed in developmentally exposed larvae, in agreement with shifts in the proteome of offspring exposed to guanylurea in the laboratory. Results of the present study indicate that current metformin exposure scenarios may be sufficient to negatively impact the fitness of developing non-target aquatic biota.

02.10.18 Differences in PM2.5 Composition, Oxidative Potential, and Developmental Toxicity Collected Across Months at Locations Throughout Tennessee

V.T. Aminone, C. Roper, University of Mississippi / Biomolecular Sciences

Differences in $PM_{2.5}$ Composition, Oxidative Potential, and Developmental Toxicity Collected Across Months at Locations Throughout Tennessee. Aminone Tonia Voke, Courtney Roper Health effects following exposure to fine particulate matter ($PM_{2.5}$) are of important concern to humans and animals, with a growing number of studies finding associations between developmental effects and $PM_{2.5}$ exposures. Oxidative stress may cause the observed developmental effects and also exacerbate co-morbidities. Oxidative stress can lead to cell and tissue damage through a variety of pathways including inflammation and apoptosis. The goal of this study is to determine the chemical composition of $PM_{2.5}$ from various locations and months and then assess the oxidative potential and developmental toxicity of these samples. Daily $PM_{2.5}$ filters from September, October, November and December for 3 locations were collected by the Tennessee Department of Environment and Conservation (TDEC) in 2014. Black carbon concentrations were determined for each filter using a Magee OT21 Sootscan at 370 nm and 880 nm, prior to extraction. Aliquots of the daily extracted $PM_{2.5}$ solutions were removed to assess oxidative potential using a Dithiothreitol (DTT) assay at 412 nm. The remaining $PM_{2.5}$ solutions will be pooled by month and location and used to conduct developmental exposures in zebrafish from 6 hours post fertilization to 5 days post fertilization. Mortality, morphology, and behavioral changes will be measured and compared between treatment and control groups. Preliminary data for black carbon concentrations in September ranged from 0.62 to 2.87 $\mu g/m^3$ at the three sampling locations (Columbia, Jackson, and Nashville, TN). The monthly average black carbon concentrations ($\mu g/m^3$) with standard deviations for each location were: Columbia (1.28 ± 0.52), Jackson (1.55 ± 0.53), and Nashville (1.68 ± 0.48). Oxidative potential and zebrafish exposures are underway and we expect to see differences in oxidative potential between days and differences between locations and months in developmental toxicity outcomes including mortality and behavioral changes. Comparisons will be made between $PM_{2.5}$ concentration and composition and the toxicity analyses. This work will highlight the differences between sampling locations and time in $PM_{2.5}$ composition, oxidative potential, and developmental toxicity.

02.10.19 Long-Term Impacts of Common Microplastic Polymers and Plastic Additives on the Proteome of Juvenile Lake Trout (Salvelinus namaycush)

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Microplastics are recognized as a major contaminant of concern, however, the long-term toxicity of microplastic polymers and their chemical additives have been poorly investigated. Particularly in regard to their impacts on culturally and economically important freshwater fish species such as Lake Trout *(Salvelinus namaycush)*. UV-stabilizers and antioxidants are common plastic additives that have been identified as contaminants of emerging concern due to their ability to bioaccumulate and act as endocrine-disrupting compounds. Still, they remain understudied, in general, and as a cocktail with microplastic particles. Here, we exposed newly hatched Lake Trout *(Salvelinus namaycush)* to a mixture of microplastics comprised of equal portions of polypropylene (positively buoyant), polystyrene (neutrally buoyant) and polyethylene terephthalate (negatively buoyant) ranging in size from 7 µm - 500 µm. The polypropylene and polystyrene were impregnated with a mixture of common UV-stabilizers (i.e. Chimassorb, Tinuvin, benzotriazole, titanium dioxide) and/or anti-oxidants (i.e. Irganox B-215, Irgafos 126). Microplastic concentrations (0, 2, 35, 315, 2669, 22465 n/L) were selected to reflect environmentally relevant freshwater concentrations with the highest concentration reflecting projections for freshwater ecosystems in the future if we continue business-as-usual. Additional controls were employed at the highest concentration which consisted of 1) microplastics alone with no additives and 2) additives only with no microplastics. Juvenile Lake Trout were exposed to microplastics and additives during yolk-sac absorption and then for an additional six weeks post initial feed for a total of 11 weeks of exposure, during important developmental stages. Whole individuals were collected for proteomic analyses at two time points, 1) once yolk-sacs had been absorbed and 2) after six weeks of feeding. Investigating proteomic alterations of both early developmental stages (analyses ongoing) will elucidate toxicity mechanisms associated with long-term exposure to microplastics and chemical additives. We also aim to answer important questions about the effects of the mixture of additive chemicals and physical microparticles. Overall, this work will inform the potential vulnerability of a significant North American freshwater fish species to the long-term exposure of microplastics and their chemical additives.

02.10.21 Later-Life Effects of Early-Life Benzo(A)Pyrene Exposure on Respiration in Zebrafish (Danio rerio)

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Benzo(a)pyrene (BaP) is a polycyclic aromatic hydrocarbon with toxicities including carcinogenicity, immunotoxicity and teratogenesis, and it is an important environmental contaminant. There is a lack of research surrounding the relationship between BaP exposure and mitochondrial bioenergetics throughout the lifespan. This study utilized early life exposures to BaP to assess later-life effects on respiration in zebrafish (*Danio rerio*). Fish were exposed embryonically to BaP, and respiration was measured at the age of 21-24 months old. Respirometry measurements were taken using a swim tunnel in order to calculate whole organismal basal respiration. An extracellular flux analyzer was used to measure oxygen consumption in the gonads, liver, heart, and brain in each fish to determine tissue-specific respiration. Fish exposed to BaP exhibited lower total organismal basal respiration compared to controls, indicating an altered basal metabolic rate for the exposed fish. The liver tissue of exposed fish showed decreased total basal respiration, mitochondrial basal respiration, and maximal mitochondrial respiration. This suggests that BaP exposure caused decreased mitochondrial function in the liver as the fish aged. The brains of BaP-exposed fish also exhibited a lower average for basal mitochondrial respiration, suggesting decreased mitochondrial function in the brain. These findings demonstrate that early life exposures to BaP can have profound later-life effects on respiration and mitochondrial function. These findings are relevant to both human and ecological health, as BaP is a ubiquitous contaminant in both the natural and built environments.

02.10.22 Thyroid Hormone System Disruption Is Life Stage Specific in Fish: The Case Study of Swim Bladder Inflation in Zebrafish

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There is broad international consensus among scientists and regulators that thyroid hormone system disruption (THSD) is of concern for environmental health. We recently described an adverse outcome pathway (AOP) network linking thyroid hormone system disruption (THSD) to impaired swim bladder inflation and reduced swimming performance in fish. The domain of applicability of these AOPs involves an important life-stage specific component. The swim bladder consists of two chambers which inflate at different time points during development. During embryonic development, inhibition of TH activation (i.e., the conversion of thyroxine to triiodothyronine by deiodinase enzymes) leads to an adverse effect on the inflation of the posterior chamber while inhibition of TH synthesis has no effect. Inflation of the anterior chamber in juvenile fish on the other hand is affected by both inhibition of TH activation and TH synthesis. The current study gains further insight into the mechanisms underlying this life stage specificity. Zebrafish embryos were exposed during specific time windows to iopanoic acid, a deiodinase inhibitor. Exposure windows were based on the different stages of swim bladder development: budding, pre-inflation and inflation phase. The budding and pre-inflation phase mark the formation of the swim bladder and the three structural tissue layers. During the inflation phase air is gulped in order to inflate the organ. Swim bladder inflation was impaired in embryos exposed during the budding and pre-inflation phase. When exposed during the inflation phase, no impaired swim bladder inflation was observed. These results suggest that the formation of the swim bladder is sensitive to THSD, whereas the inflation process itself is not. Further analyses, including gene expression analysis, are being performed to understand how deiodinase inhibition affects swim bladder development. Comprehending the life stage specificity of THSD is not only important for swim bladder inflation but for other endpoints as well, including eye development, pigmentation and fin development. Our results contribute to a more comprehensive understanding of the mechanistic basis through which THSD affects different endpoints. This could then aid in the further development of THSD screening methods.

02.11 Pelagic and Benthic Harmful Algal Blooms: The Detection, Fate, Effects, Monitoring, and Management of Blooms

02.11.01 Prevalence and Persistence of Microcystin in Shoreline Lake Sediments and Porewater, and Associated Potential for Human Health Risk

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Midlatitude waterbodies are experiencing increased cyanobacteria blooms that necessitate health advisories to protect waterbody users. Although surface waters may contain cyanotoxins such as microcystin (MC), at concentrations that pose potential public health risks, little is known about MC contamination of shoreline sediments. Based on growing evidence that lake and reservoir sediments can accumulate MCs, we hypothesized that shoreline sediments (i.e., recreational beaches) may accumulate MCs and thereby pose a potential health risk to recreational users even if people stay out of contaminated water. We sampled nearshore surface water, shoreline sediment, and porewater from seven Washington State, USA, lakes/reservoirs recreational beaches to determine MC presence/absence during or immediately following cyanobacteria blooms. We found MCs in shoreline sediments at all waterbodies using ELISA and LC-MS/MS. MC concentrations in shoreline sediments and porewaters persisted for 20 days following dissipation of cyanobacteria blooms when MC concentrations were near analytical reporting limits in corresponding surface waters. A human health risk assessment based on potential MC exposure through incidental ingestion of porewaters and sediments found, even when very high MC concentrations occur in surface waters (i.e., >11,000 ppb), estimated ingestion doses are below MC World Health Organization tolerable daily intake and U.S. Environmental Protection Agency's risk reference dose. Our findings suggest MCs in Washington State recreational beaches in 2018 did not present a significant human health risk, but the findings provide evidence of an exposure pathway for MCs via shoreline sediment/porewater exposure.

02.11.02 Cyanobacterial Toxins in Recreational and Intake Waters Using a Targeted UPLC/MS/MS Method

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Liquid Chromatography/Tandem-Mass Spectrometry (LC/MS/MS) is a powerful tool for the analysis of various analytes in a wide variety of matrices. What is especially attractive about LC/MS/MS is its sensitivity and selectivity. Various cyanobacterial algae that produce toxins, microcystins being one of the more well-known, represent an emerging class of algal toxins of concern to both the recreational and drinking water industry. In this paper we investigate the use of smaller column packing (sub 2µm particles) to monitor for many of these toxins using a generic gradient method. Specifically, microcystins, anatoxin-a, cylindrospermopsin as well as newer toxins, such as euglenophycin, anabaenopeptins and micropeptins using Ultra-Performance Liquid Chromatography (UPLC®) combined with tandem mass spectrometry will also be tested. We investigated the analysis of a wide variety of freshwater samples from throughout the United States and cultured strains from the lab. Examples showing additional toxins detected in freshwater samples will be presented including data from the recent blooms in the Ohio River, North Carolina, Florida, California and other lakes in the US and elsewhere. New work using a novel ionization technique (called UniSpray) will be shown for toxin analysis and compared to standard electrospray ionization. Comparison of blooms from a similar source over several years will also be discussed with similarities shown in the targeted analysis over that time.

02.11.03 Are Cyanotoxins a Health Risk for Athletes Exercising Around an Urban Lake in Mexico City?

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The incidence of *blooms* of cyanobacteria in urban lakes in Mexico City has increased in recent years. The most common genus in freshwater bodies is *Microcystis*, and some species are microcystins producers; these toxins are hepatotoxic for humans. There are records of massive *blooms* of these organisms in the Cuemanco Olympic Rowing and Canoeing Track (ORCT) in Mexico City, a place where lots of people go daily to perform sports activities, however, studies in the area are not routine. Therefore, the objective of this work was to evaluate the risk for athletes of the presence of microcystin-producing cyanobacteria in the ORCT. To this end, surveys were conducted with ORCT athletes, as well as toxicity bioassays with lettuce seeds (*L. sativa*); these were exposed to an ORCT water extract containing cyanobacteria. Significant decreases were observed in the germination and elongation of the root and hypocotyl. The exposure data obtained from the surveys suggest that the presence of these toxins represents a health risk to those who attend daily and for prolonged periods, mainly due to the chronic effects that microcystins can generate in humans. The limited information on the exposure routes suggests that not only athletes who train within the ORCT are exposed to the effects of the toxins, but also those who perform physical activity in the surroundings, due to the possible presence of microcystins in aerosols. The respiratory route is poorly studied, but equally important for assessing health risk in athletes and the general population.

02.11.04 Develop Measures of Cyanotoxins and Other Algal Toxins Associated with Ecological Harm Using Aquatic Test Organisms

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There is a lack of information to estimate safe exposure levels for aquatic life to natural toxins produced by cyanobacteria and the freshwater invasive microalgae *Prymnesium parvum*. The uncertainty in concentrations and purity of standards for cyanotoxins, as well as their high cost, challenge their use for acute and chronic toxicity tests. An alternative approach was tested, using cultures of cyanobacteria and algae to generate toxins. In this study, we have developed laboratory cultures of toxin producers *Microcystis aeruginosa* UTEX B2666 (microcystin), *Anabaena flos-aquae* SAG 30.87 (anatoxin), *Aphanizomenon flos-aquae* PCC7905 (cylindrospermopsin), and *Dolichospermum circinale* CS-337 (saxitoxin) as well as non-toxin strain of A. *flos-aquae* and *P. parvum* UTEX 2797 (prymnesium). Tests were conducted with intracellular toxins obtained by removing cells from culture media and lysing them. Microcystins concentrations varied if only cell number and age of culture were tracked, with 1.75 X 10^6 cells/ml giving a microcystin concentration of 885 ug/L, while 4.16 X 10^6 cells/ml yielded 37 ug/L. Acute tests conducted with *Ceriodaphnia dubia*, *Neocloeon triangulifer*, *Hyalella azteca,* and larval *Pimephales promelas* using microcystins, did not cause any lethality different from the control at concentrations as high as 74 ug/L. However, the non-toxin-producing strain of *A. flos-aquae* caused mortality greater than the controls to *N. triangulifer.* 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 ug/L, respectively. For *P. parvum* LC50s were 4.94 X 10^6 cells/ml for *C. dubia* and 1.22 X 10^6 cells/ml *N. triangulifer.* A method for measuring prymnesin is under development. Due to the variability in getting consistent intracellular toxin levels, an approach was taken to grow all of the 5 cyanobacteria cultures up to a stationary phase then test for toxin concentrations using ELISA methods. This was done in 250 ml cultures 3 times. Then cultures were increased to 4-liter volumes, then the isolated intracellular toxin was tested in a chronic format. Prymnesin was also tested in this same manner. Results of the culture methods used for all 5 of the cyanobacteria will be presented as well as the chronic or short-term growth results for microcystin, anatoxin, cylindrospermopsin, and saxitoxin.

02.11.05 Evaluating Airborne Harmful Cyanobacterial Bloom Compounds As a Public Health Concern in the Chowan River, NC

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In addition to obvious negative effects on water quality, recent findings suggest that harmful cyanobacterial blooms (CHABs) impact air quality via primary spray aerosol emissions carrying cyanobacterial cells and cyanotoxins. The inhalation of microcystin (MC), a potent liver cyanotoxin, elicits a more cytotoxic response compared to other exposure routes. People are most likely to come into contact with aerosolized CHAB toxins during recreational water activities, but these aerosols can persist into communities onshore. Thus, the objective of our study was to quantify CHAB toxins and examine CHAB DNA sequence diversity in the airshed of the Chowan River, North Carolina, a eutrophic estuary facing recurrent CHABs. Our field campaign (Summers 2020 and 2021) was the first to evaluate inhalation as a potential exposure pathway to MC in NC. From June to Oct., several two-week integrated, daytime $PM_{2.5}$ (particulate matter with diameters < 2.5 µm) samples were collected using medium-volume samplers (*Tisch Environmental*). Offline analytical methods were applied to examine DNA and MC in water and aerosol samples. Physicochemical water parameters, ambient $PM_{2.5}$ mass concentration, and meteorological conditions were also recorded in parallel. In 2020, CHAB genera dominated the algal community biomass (~45%), but maximum toxin concentrations and toxin encoding genes (*mcyA*) in water samples were low.

02.11.06 Deployable and Retrievable Photocatalyst Composite Structures to Reduce Harmful Algal Blooms and Toxins, Enabled by Additive Manufacturing (3D Printing)

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Harmful algal blooms (HABs) induce damage to natural resources, recreation, and health. Traditional chemical controls involve addition of non-specific, broadcast algaecides (e.g., copper-based compounds, peroxides, etc.) into surface waters that may cause legacy contamination and impact non-target organisms. Research and development into innovative and sustainable management solutions are needed. Photocatalytic TiO_2 nanoparticles offer a sustainable treatment strategy for HABs and released algal toxins by generating reactive oxygen species under natural solar radiation or artificial UV light. The associated free radical effects are localized, short-lived and not expected to have adverse implications on nearby non-target organisms. However, free TiO_2 nanoparticles rapidly agglomerate and settle out of the photoactive zone and are not retrievable from the environment once deployed. This investigation proposes a unique solution by compounding TiO_2 nanoparticles (76% anatase) into transparent polylactic acid (PLA) to allow light transmission. The thermoplastic polymer PLA is derived from natural materials (e.g., sugarcane, corn) and has greater biocompatibility than petroleum-based polymers. The PLA-TiO_2 was 3D printed using a desktop fused filament fabrication printer into high surface area deployable and retrievable lattice geometries and tested for efficacy to reduce *Microcystis aeruginosa* cell density (measured by optical and hemocytometry methods) and total microcystin toxin (ELISA method). Benchtop experiments indicated greater than 90% reduction in methylene blue (1 mg/L) under ReptiSun® 5.0 bulbs (2 W/m^2 UV; 280-400 nm), confirming the printed PLA-TiO_2 composites (23% TiO_2 w/w) retained comparable efficacy to free TiO_2 after 10 hours light exposure. While cells grew in the light condition lacking photocatalyst (from $4x10^6$ up to $8x10^6$ cells/mL), presence of the 3D printed photocatalyst composites significantly ($p < 0.05$) reduced cell density by more than 85% (< $1x10^6$ cells/mL), resulting in optically clear test solution within 48 hours. The PLA-TiO_2 composites also reduced microcystin from 30 µg/L to below detection limits. Further investigations of this on-demand, retrievable, and reusable additively manufactured technology include photocatalyst loading optimization, determination of the kinetics of HABs treatment efficacy, and scale-up from microcosm to mesocosm to field applicability.

02.11.07 Clearly Contaminated: BMAA and Non-Bloom Forming Cyanobacteria in Oligotrophic Lakes

K. Low, University of New Hamsphire

Beta-methylamino-L-alanine (BMAA) is a widespread cyanotoxin that may pose health risks to humans and wildlife. Although most cyanotoxin research focuses on nutrient-rich systems that support persistent cyanobacteria blooms, BMAA is also prevalent in oligotrophic systems that are less often afflicted by visible blooms. As part of a larger effort to track the movement of BMAA through aquatic food webs, this study aimed to identify sources of BMAA in two oligotrophic lakes that lack surface blooms. Preliminary results suggest both lakes supported bloom-forming cyanobacteria genera in low densities. Fluorometric measurements of phycoerythrin (PE) could indicate an abundance of picocyanobacteria. However, baseline PE levels have not been established in Wyoming lakes, so additional analysis of picocyanobacteria is needed. Cyanobacteria biomass, as estimated by PE, was positively associated with BMAA and was significantly higher in the benthic and littoral areas than in the pelagic zone. The community composition of the benthos differed significantly from that of the pelagic zone. However, these differences appear to be driven largely by diatoms rather than cyanobacteria. Therefore, toxicity appears to be more strongly related to biomass than to community composition. While preliminary, these results suggest that non-bloom forming cyanobacteria are a significant source of BMAA in oligotrophic lakes in Wyoming. Based on these results, we can infer that BMAA production in eutrophic systems may be high even in the absence of surface blooms, as picocyanobacteria and non-bloom forming benthic cyanobacteria may be present in even higher densities in eutrophic systems.

02.11.08 Nutrient Dynamics of Microcystin and Anatoxin Production in the Western Lake Erie Cyanobacterial Harmful Algal Blooms

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Cyanobacterial harmful algal bloom (CyanoHAB) proliferation is a global problem impacting ecosystem and human health. Western Lake Erie (WLE) typically endures two highly toxic CyanoHABs during summer: a *Microcystis* spp. bloom in Maumee Bay that extends throughout the western basin and a *Planktothrix* spp. bloom in Sandusky Bay. Recently, USA and Canada agreed to a 40% phosphorus (P) load reduction to lessen the severity of the WLE blooms. To investigate P and nitrogen (N) limitation of biomass and cyanotoxin production in WLE CyanoHABs, we conducted *in situ* nutrient addition and 40% dilution microcosm bioassays in June and August 2019. During the June Sandusky Bay bloom, biomass production as well as hepatotoxic microcystin and neurotoxic anatoxin production were N and P co-limited with microcystin production becoming nutrient deplete under 40% dilution. During August, the Maumee Bay bloom produced microcystins under nutrient repletion with slight induced P limitation under 40% dilutions, and the Sandusky Bay bloom produced anatoxin under N limitation in both dilution treatments. The results demonstrate the importance of recognizing spatiotemporal heterogeneity in nutrient limitation in aquatic systems and to properly combat cyanotoxin production in WLE, both N and P reduction efforts should be implemented in the WLE watershed.

02.11.09 The Ecotoxicology of Microcystins in Freshwater Environments: Prospects for Future Research

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The increasing frequency and intensity of harmful (toxin-producing) algal blooms (HABs) has emerged as one of the most significant environmental issues threatening the health of freshwater aquatic ecosystems. Microcystins are a family of more than 250 structurally similar hepatotoxins produced by species of freshwater cyanobacteria, primarily the alga *Microcystis aeruginosa*. Understanding the toxicity of these toxins, particularly microcystin-LR, on freshwater aquatic biota has constituted a key focus of our research in recent years. In this presentation, drawing on work conducted by the co-authors and similar work published in the literature, we review the state of the science concerning the ecotoxicology of microcystins in aquatic invertebrates and fish, incorporating both whole-organism and sub-organismal (molecular) responses, to identify important areas and key hypotheses that can form the basis of future research.
To date, our studies have included invertebrates (*Daphnia magna* and *Hexagenia* spp.) and fish (*Oncorhynchus mykiss*, *Salvelinus namaycush*) selected to represent both pelagic and sediment environmental compartments. While our assessments include acute toxicity determinations, we have largely focused chronic and long-term (multi-generational) toxicity, differentiating between intra-and-extracellular toxin exposure (both of which may occur over the life span of a bloom), and identifying causal linkages between whole organism and molecular responses. This will provide a much stronger basis on which to characterize potential risks to organisms exposed to HABs, and on which to establish/refine water quality criteria and fish consumption guidelines.

02.11.10 You Aren't What You Eat: Preliminary Data Counter Biomagnification of BMAA in Freshwater Lake Food Webs

K. Low, University of New Hamsphire

Beta-methylamino-L-alanine (BMAA) is a widespread toxin that may contribute to the development of neurodegenerative diseases in humans and wildlife. Because BMAA is produced by cyanobacteria, most research focuses on highly-eutrophic systems with recurring cyanobacteria blooms. However, oligotrophic systems that lack obvious surface blooms are also known to produce low levels of BMAA, and predators feeding high in these food chains can accumulate high BMAA concentrations relative to background levels. Here, we investigate the biomagnification of BMAA as a possible explanation for the accumulation of BMAA in top-predators occupying oligotrophic systems. Preliminary results suggest that non-bloom forming cyanobacteria are important sources of BMAA, with the highest concentrations of both cyanobacteria and BMAA found in shallow and benthic regions in these nutrient-poor lakes. With a strong link to phytoplankton biomass, BMAA toxicity in the environment was 1 – 4 orders of magnitude lower than even the lowest trophic levels (plankton), depending on the sample type. We found little evidence to support further concentration of BMAA levels in the food web, with only BMAA concentrations in Red-Sided Shiners (*Richardsonius balteatus*) surpassing those found in plankton samples. BMAA concentrations in Columbia Spotted Frogs (*Rana luteiventris*) and Common Loons (*Gavia immer*) were lower than those found in plankton. Although trophic positions have yet to be confirmed through stable isotope or gut content analysis, BMAA does not appear to biomagnify through the food web in these oligotrophic systems. Future research should focus on alternative exposure pathways that could account for the accumulation of BMAA in top-predators, such as the inhalation of aerosolized toxins.

02.11.12 Qualitative and Quantitative Assessment of Epiphytic/Benthic Algae Using Artificial Substrate Methods in the Finger Lakes, New York

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In recent years, harmful algal blooms (HABs) have occurred on all eleven of New York's Finger Lakes and are a growing concern for environmental and human health. Epiphytic/benthic HABs have received less attention than their planktonic counterparts, and very little is known regarding their cyanobacterial community, ecology, and risk to the Finger Lakes ecosystem. This study aims to develop sampling and assessment methods for epiphytic/benthic cyanobacteria in two mesotrophic Finger Lakes using artificial substrate, genetic and toxin analyses, and pigment extraction. Biofilm, Solid Phase Adsorption Toxin Tracking (SPATT), and water samples will be collected throughout the growing season to address these questions in the presence and absence of a bloom. We hypothesize that the attached cyanobacterial communities and their toxin production will differ based on physiochemical conditions of the water, duration of incubation in the lake, and position in the water column. This study will provide necessary data to inform local lake associations and monitoring programs of proper sampling and monitoring techniques for epiphytic/benthic growth and identify the relative risk posed by dominant cyanobacteria species in the Finger Lakes.

02.12 UV Filters in Aquatic Ecosystems

02.12.01 Assessing Ahr-Mediated Toxicity of Benzotriazole Ultraviolet Stabilizers (UV-P, UV-9, UV-090) Using In Vivo Embryo Microinjection and an In Vitro AhR Activation Assay

H. Johnson, University of Lethbridge / Biological Sciences; J. Doering, University of Lethbridge; J. Dubiel, University of Lethbridge / Department of Biological Sciences; Z. Lu, University of Quebec, Rimouski / Institut des Sciences de la Mer de Rimouski; S. Wiseman, University of Lethbridge / Biology

Plastics contain various chemical compounds that enhance the longevity and quality of the product. Specifically, the addition of benzotriazole ultraviolet stabilizers (BUVS's) helps counter the degradation and discoloration of plastic materials from UV radiation. Due to improper disposal of plastics, chemicals such as BUVS's can leach and into aquatic ecosystems. As a result, BUVS's are ubiquitously detected in the aquatic environment and biota, causing concern for the health of fishes and other aquatic wildlife. There is currently only limited toxicity data for BUVS's, but these studies suggest that certain BUVS's might dysregulate the aryl hydrocarbon receptor (AhR) causing early life-stage toxicity in fishes. Therefore, there is need for a more comprehensive analysis of the effects caused by exposure to BUVS's and risks posed by these chemicals. The present study exposed embryos of zebrafish (*Danio rerio*) to precise serial doses of three priority BUVS's, namely 2-(benzotriazol-2-yl)-4-methylphenol (UV-P), 2-(Benzotriazol-2-yl)-4-methyl-6-prop-2-enyl-phenol (UV-9), or 2-[3-(2H-benzotriazol-2-yl)-4-hydroxyphenyl]ethyl methacrylate (UV-090) through microinjection. Toxicity of each priority BUVS was assessed by recording early life-stage malformations and mortality. Embryos exposed to BUVS experienced mortality in a dose dependant manner, with the most potent chemical tested being UV-P, which had a median lethal dose (LD50) of 4772 ng/g-egg. Contrary to this, UV-090 was the least potent chemical tested with a LD50 of 56292 ng/g-egg. UV-P, UV-9, and UV-090 were less potent in all cases than TCDD, the prototypical AhR agonist, with relative potencies of 0.0005, 0.0002, and 0.00005, respectively. The extent to which each of the BUVS activates the AhR was determined with a luciferase reporter gene (LRG) assay using COS-7 cells transfected with the AhR of zebrafish and two native North American fish species, namely brook trout (*Salvelinus fontinalis)* and lake sturgeon (*Acipenser fulvescens*). UV-P and UV-9 activated the AhR of each species, but UV-090 did not activate the AhR of any species at the greatest tested dose. The rank order of sensitivity for both BUVSs was zebrafish > lake sturgeon > brook trout. Results from this study will guide more objective assessment of risks posed by BUVS's for the protection of diverse fish populations.

02.12.02 The Effects of UV-Stabilizers, UV-9 and UV-090, on Oocyte Maturation in Zebrafish (Danio rerio)

Y. Raza, University of Lethbridge / Department of Biological Sciences; J.G. Miller, University of Lethbridge / Biology; J. Doering, University of Lethbridge; S. Wiseman, University of Lethbridge / Biology

Benzotriazole UV-Stabilizers (BUVSs) are chemicals that work to protect against UV degradation. BUVSs are found in a variety of industrial materials and consumer products, such as plastics, paints and waxes. Although studies have demonstrated widespread contamination of aquatic environments by BUVSs, little is known about their toxicological effects. This study aimed to explore whether BUVSs, UV-9 and UV-090, impair reproduction of zebrafish (*Danio rerio*) by inhibition of oocyte maturation. Effects of BUVSs on oocyte maturation were determined using both *in vitro* and *in vivo* assays. To assess effects of *in vitro* exposure, stage IV oocytes were excised from sexually mature female zebrafish and exposed to various concentrations of each BUVS, followed by stimulation of maturation by exposure to maturation-inducing hormone. When exposed to UV-9 *in vitro*, there was significant inhibition of oocyte maturation at the 200µg/L concentration, compared to the control. In the UV-090 exposure, there was significantly less maturation of oocytes exposed 200, 2000, or 20000µg/L concentrations, compared to the control. To assess effects of *in vivo* exposure, sexually mature female zebrafish were fed a diet of 25 (low), 125 (medium) or 625 (high) ng BUVS/g food. Following 10-days of exposure, stage IV oocytes were excised to assess maturation in response to maturation-inducing hormone. There was significantly less maturation of oocytes from fish exposed to UV-9 at 125 and 625ng/g food, when compared to the control. However, there was no inhibition of oocytes from fish exposed to all concentrations of UV-090. Overall, results suggest that UV-9 impairs oocyte maturation *in vitro* and *in vivo*; whereas UV-090 may be a less potent inhibitor of oocyte maturation because inhibition was only observed *in vitro*. Studies to assess the molecular basis of effects of UV-stabilizers on oocyte maturation are in progress and will be presented. Also, studies of effects of BUVSs on reproductive capacity and to determine whether assays of oocyte maturation are predictive of reproductive performance are in progress and will be presented. This study increases understanding of the possible toxicological effects of exposure to BUVSs in fishes and other aquatic wildlife.

02.12.03 Chronic Effects of Benzophenones (BPs) on Fathead Minnow

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Benzophenones (BPs) are ultraviolet filters found in sunscreen formulas and other personal care products, as well as in many industrial products. Degradation of benzophenones in wastewater treatment is variable, and so concentrations of up to 0.7 µg/L have been detected in municipal wastewater effluents (MWWEs). The current study investigated the chronic effects of three benzophenones (BP-4, BP-1, and BP-3) in early life stages of the fathead minnow (*Pimephales promelas*). Eggs and larvae were exposed to concentrations of BP-4 at nominal concentrations of 0, 0.1, 1, 10, 100, 1,000 and 10,000 µg/L in a daily static-renewal set-up. There were 12 replicates of control exposure beakers and 4 replicates of each BP-4 exposure beaker, with each replicate beaker containing 20 fathead minnow embryos/larvae. The two lowest benzophenone exposure concentrations represent approximate river and MWWE concentrations of these compounds. Mean (s.d.) measured concentrations of BP-4 were 0.143 (0.022), 1.26 (0.10), 12.0 (0.4), 110.5 (4.0), 1097 (48), and 11,469 (1076) µg/L, which were on average 115 % of nominal exposure concentrations. Concentrations of BP-4 in the control exposure beakers were always non-detectable. Over the 21-d exposure (5 days in the embryo, and 16 days post-hatch, dph), BP-4 did not affect survival or growth of the larval fish. Hatch success, time to hatch, deformities in hatched fry, and survival until 9 and 16 dph were similar across all treatments (0.340 < ANOVA p < 0.981). Growth (wet weight, length, and condition factor) assessed at 9 and 16 dph-was not affected by BP-4 up to 10,000 µg/L (0.735 < ANOVA p < 0.996). Preliminary data from rangefinder experiments that exposed a limited number of fish to BP-1 and BP-3 (oxybenzone) suggest these benzophenones do affect survival, growth, and deformities but only at high exposure concentrations. These exposures show that the three tested benzophenones at environmentally-relevant concentrations (up to 1 µg/L), do not cause any observable negative effects in this commonly-used lab fish species.

02.12.04 Use of an Eco-Epidemiology Approach to Assess Potential Risks of Natural and Anthropogenic Factors, Including UV Filters, to Coral Community Status in Hawaii

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In the past few years, questions have been raised regarding the environmental safety of some UV filters used in personal, skin care and beach products to corals. In some cases (e.g., Hawaii, Key West, Palau) regulatory actions have been precautionary, leading to bans. Unfortunately, no regulatory authority has explicitly attempted to quantify the impacts of UV filters on corals relative to other forms of pollution or environmental factors. Eco-epidemiology is a methodology that considers species and communities as affected by complex combinations of multiple physical, chemical, and environmental conditions over time. This study assembled a large set of natural and human influenced factors (including potential risks of UV filters) along with coral cover data for the Hawaiian Island of Oahu to assess the potential adverse effects of UV filters on corals within the context of other factors. All data were spatially analyzed using a geographic information system. Principal component analyses were used to determine the relationships of coral ecological data to natural and anthropogenic factors. Results indicated that coral cover could be explained via species diversity and abundance. These aspects and all other factors were then correlated to each other to determine if some factors could act as proxies for each other (e.g., beach visitors as a proxy for UV filters) and if any factors appeared to be highly related to coral diversity and abundance. Wave power, sea surface temperatures and sedimentation were shown to be highly correlated to coral ecological status. Statistically significant regressions for coral diversity included temperature anomalies and wave power, both of which addressed the vast majority of the variance. UV filters did not significantly contribute to decreases in coral diversity. Regressions for coral abundance indicated that sewage effluent and sedimentation were more significant than UV filter hazards. Hence, it appears that UV filter hazards do not significantly address reduced coral diversity and abundance whereas wave power, temperature and sedimentation appear as the dominant factors affecting coral ecological status.

02.12.05 Distribution and Fate of UV Absorbents and Industrial Antioxidants in the St. Lawrence River

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Ultraviolet absorbents (UVAs) and industrial antioxidants are contaminants of emerging concern. Limited knowledge is available for the distribution and fate of these contaminants in the environment. In this study, we investigated the distribution and partitioning of UVAs (including UV stabilizers and UV filters, UVFs) and industrial antioxidants (including synthetic phenolic antioxidants and secondary aromatic amines) in surface water, sediment, and various tissues of two predator fish species from the St. Lawrence River, Quebec, Canada. The St. Lawrence River is a large, high flow river that flows over 3000 km before discharging into the Atlantic Ocean. Montreal is the second largest city in Canada by population and its wastewater treatment plant (WWTP), which discharges primary treated effluent into the St. Lawrence River. The water collected downstream of the WWTP point of discharge in the river showed higher levels of various UVFs (total UVFs: 30-318 ng/L), 2,6-di-tert-butyl-1,4-benzoquinone (BHT-Q; < MQL- 260 ng/L) and diphenylamine (DPA; < MQL-24 ng/L) compared with upstream sites, suggesting that the WWTP may be a source of contamination. However, there were no significant spatial differences in the levels of these contaminants in the northern pike (*Esox lucius*), possibly due to the relatively short biological half-life of these contaminants (0.2-10 days) in fish. The liver was the dominant tissue in the northern pike to accumulate these contaminants. 2-6-di-tert-butyl-4-methylphenol (BHT) was detected in fish brains, indicating that this contaminant may cross the blood-brain barrier. Lake sturgeon (*Acipenser fulvescens*) were only collected from upstream of the WWTP (Lake St. Louis). Lake sturgeon and northern pike showed different profiles for several target contaminants, indicating species-specific accumulation processes. 2-(2H-benzotriazol-2-yl)-4,6-ditertpentylphenol (UV328) in the muscle of lake sturgeon (< MQL-117 ng/g ww) was higher than most fish samples from the Great Lakes of North America and the northern pike in the present study, indicating potential sources of UV328 close to the Lake St. Louis or unique accumulation processes of UV328 in these benthic fish. More research is warranted to elucidate the toxicokinetics and risks of these emerging contaminants in the aquatic environment.

02.12.06 Freshwater Aquatic Exposure Modeling and Environmental Safety Assessment of UV Filters

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Organic ultraviolet (UV) filters are used in cosmetic and personal care products (CPCPs), including over□the□counter sunscreens, due to their ability to absorb solar radiation. When CPCP ingredients are washed down the drain, they can then enter freshwaters that receive wastewater treatment plant effluents. In this presentation a framework to conduct down-the-drain environmental safety assessments for CPCP ingredients is presented. The framework is demonstrated though a case study of key UV filters used in sunscreens and CPCPs in the United States. Exposure was characterized using iSTREEM®, a spatially resolved aquatic exposure model developed for chemicals disposed of down-the-drain. iSTREEM® provides a comprehensive exposure assessment through the consideration of spatial variability in emissions, wastewater treatment, flow, and loads transported from upstream to estimate CPCP concentrations in receiving waters across the conterminous United States. The result is a predicted environmental concentration (PEC) distribution representative of spatial variability in conditions across the region. A review of available hazard data was used to derive a predicted no□effect concentration (PNEC) using aquatic toxicity data and assessment factors. A safety assessment was conducted by comparing the PEC distribution with the PNEC. The results indicate that freshwater exposure stemming from down-the-drain emissions to the case study UV filters is of low concern as the PNEC does not exceed 90th percentile PEC. These results are instrumental in demonstrating the environmental safety of key organic UV filters in the U.S. freshwater environment and will help prioritize future work.

02.12.07 Temporal Variations of UV Filter Concentrations at a Recreational Sandbar in Biscayne Bay, Florida

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In recent years, the UV filter oxybenzone has come under both scientific and popular media attention due to its reported endocrine-disrupting effects on corals and other marine organisms. It is important, however, to understand not only its toxicological effects but also its prevalence and origins in the marine ecosystem. Therefore, this study aims to determine the correlation of environmental oxybenzone concentrations with visitor activity, tidal levels, and recent rain events, as well as overall trends over the course of a summer. The site chosen for this study is the Haulover Sandbar, a popular recreational sandbar located in a semi-enclosed arm of northern Biscayne Bay. This site was chosen due to the large numbers of visitors to the site on weekends and holidays and its relatively sheltered location. Since previous experiments have shown that oxybenzone undergoes indirect photodegradation in the presence of natural organic matter found in northern Biscayne Bay, two other UV filter compounds- avobenzone and octocrylene- are also tested for. Both are commonly found in sunscreen formulations together with oxybenzone in the United States and should therefore be present at higher levels at times when more sunscreen has entered the water. Water samples are taken at the sandbar just below the surface at times of varying visitor and water levels. Concentrations of all three UV filters are determined via LC/HRMS using a Thermo Q-Exactive orbitrap mass spectrometer with on-line solid phase extraction and heated electrospray ionization source in positive mode. Previous studies on UV filters in Biscayne Bay have shown concentrations of oxybenzone in 26% of surface samples tested at concentrations of up to 31 parts per trillion. It is hypothesized that environmental concentrations of UV filters will correlate directly with the number of visitors at the sandbar, since sunscreen worn during marine recreation is expected to be the major environmental source of UV filters. Another potential source of UV filters in the environment is runoff from urban river and canal systems, which would increase after periods of significant rainfall. Tidal action is also hypothesized to be a major factor due to large exchanges of water between the enclosed and heavily urbanized Biscayne Bay and the open Atlantic Ocean through the nearby Haulover Inlet.

02.13 Aquatic Toxicology, Ecology and Stress Response

02.13.01 A Novel Mechanism of Stressor Avoidance in Pelagic Fish Embryos

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Pelagic fish embryos are thought to float in or near surface waters for the majority of their development and are presumed to have little to no control over their mobility. However, we have shown that pelagic embryos of marine fish possess an apparently adaptive UV avoidance response. Stressors occurring in surface waters, such as increased temperature, cardiotoxic oil slicks and ultraviolet radiation (UVR), pose a serious threat to the fitness and survival of the vulnerable early life stages of fish. This is especially important when considering the interactive effects of stressors, such as photo-induced toxicity—a phenomenon in which the toxicity of a compound is amplified after exposure to certain wavelengths of light. Therefore, a mechanism in which embryos can alter buoyancy and thus control their vertical position in the water column may be indispensable to the sustainability of these fisheries. Co-exposure to crude oil and other environmentally relevant stressors, such as increased temperatures and UVR, induced an early onset of negative buoyancy in developing mahi-mahi (*Coryphaena hippurus*) embryos. Further, recovery of positive buoyancy was observed once the exposure period was completed and all stressors had been terminated, indicating this response is highly dynamic and not solely pathological. Specific gravity of mahi-mahi embryos exposed to different environmental conditions was measured using density gradient columns. The Connectivity Modeling System was employed to estimate how changes in specific gravity translated to differences in the position and vertical distribution of embryos in nature. Increased specific gravity in UV-exposed embryos resulted in deeper vertical distributions, reduced cumulative UV exposures, and reduced mortality compared to their control counterpoints with no UV exposures. The finding that pelagic embryos regulate buoyancy following exposure to certain stressors may have significant implications for how we perform and interpret future toxicity tests. This research was made possible by a grant from The Gulf of Mexico Research Initiative. Grant No: SA-1520; Name: Relationship of Effects of Cardiac Outcomes in fish for Validation of Ecological Risk (RECOVER).

02.13.02 A Second-Tier Screening-Level Bioassay Methodology to Evaluate Ecologically Relevant Patterns of Pesticide Exposure to Daphnia magna

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Pesticides are subject to atmospheric and hydrolytic transport from sites of application to aquatic ecosystems. Across the landscape, concentrations in surface water can vary spatially and temporally according to seasonal use practices. Standardized bioassays can provide a first-tier screening-level understanding of aquatic receptor acute and chronic toxicity. However, these bioassays do not address ecologically relevant patterns of exposure that may impact fitness and survival within and across generations. Impact on aquatic receptors should consider the pulse magnitude, duration, and frequency on a biologically relevant time scale that can be defined by toxicant depuration rates and organismal time to recovery or adaptation. In addition, exposures may result in changes in gene expression that impact fitness and survival in subsequent generations. Also of concern are patterns of exposure that span multiple generations. We report a second-tier, screening-level bioassay methodology to evaluate ecologically relevant patterns of pesticide exposure. We employed a multi- and trans-generational bioassay spanning 4 generations of *Daphnia magna* to investigate the effect of chronic exposures to chlorpyrifos (CPF), an organophosphate insecticide that elicits neurotoxic effects in aquatic organisms by inhibiting acetylcholinesterase, prolonging nerve transmission, and resulting in neurotoxic symptoms and death at high doses. The multi-generational assay consisted of 4 consecutive 21d bioassays using progeny from the previous assay for each successive generation. In the trans-generational assay, only the parent (F0) generation was exposed. For both assays, survival and reproduction were assessed across treatments and generations.Case-study results indicated that 1) following continuous CPF exposure at ecologically relevant concentrations to 4 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 parent (F0) generation did not result in treatment effects in the unexposed F1, F2, and F3 generations in the apical endpoints of survival and reproduction.Analyzing aquatic receptor response over multiple generations that overlap with likely patterns of pesticide exposure can provide a second-tier screening-level understanding of a population's response to ecologically relevant exposure scenarios.

02.13.03 Acute and Chronic Toxicity of Ammonia and Nitrate to a Native Freshwater Mussel (Arkansas Brokenray, Lampsilis Reeveiana) From the Buffalo National River, Arkansas

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Historical water quality data have raised concern that nitrogen compounds (e.g., ammonia, nitrates) may be affecting water quality in the Buffalo National River (BUFF) in northern Arkansas. Degradation of the water quality of this system is likely the result of combined sources including recreational use, runoff from nearby agricultural pastureland, aging private wastewater treatment systems, erosion, and a concentrated animal feeding operation. The objective of this study was to evaluate acute and chronic toxicity of ammonia and nitrate to a native freshwater mussel (Arkansas Brokenray, *Lampsilis reeveiana*) from the BUFF. Acute (96-h) and chronic (4- and/or 12-week) toxicity tests were conducted with juvenile mussels at 20 °C in diluted well water (pH ~8.2, hardness ~100 mg/L as $CaCO_3$). Preliminary results from ammonia tests indicated that the acute EC50 value for survival was 11 mg N/L and chronic EC20s for biomass (total dry weight of surviving organisms in a replicate) were 0.23 mg N/L in 4- week exposure and 0.12 mg N/Lin the 12-week exposure. For nitrate, the acute EC50 was 707 mg NO_3-N/L and chronic EC20 for biomass was 31 mg NO_3-N/L in 4-week chronic tests. These results provide the toxicity thresholds of ammonia and nitrate that can be used by resource managers to protect Arkansas Brokenray and other mussels in BUFF. Furthermore, these data in combination with other published values, can be used in the development and refinement of environmental guidance values for ammonia and nitrate. Currently, the data are being used in parallel with in-situ field data and laboratory effluent studies to better understand the potential role of nitrogen compounds in freshwater mussel declines in the BUFF and other protected natural systems.

02.13.04 Acute Toxicity of Sodium Chloride and Sodium Sulfate to Different Freshwater Organisms in a Low Hardness Water

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Chloride (Cl) and sulfate (SO_4) are a common surface water contaminant originating from sources such as municipal wastewater treatment plant effluent, fertilizers in runoff during overland flow events, and discharges associated with energy and mineral extraction. The United States Environmental Protection Agency's current national water quality criteria (WQC) for Cl and state water quality standards (WQS) for SO_4 have been developed based on toxicity data from laboratory tests conducted in hard and moderately hard waters. However, the toxicity of Cl and SO_4 are likely influenced by water hardness. Many ecoregions in the Southeastern United States have median hardness values in a soft water range (0 to 60 mg/L as $CaCO_3$) where the toxic effect of these ions may be greater. We conducted acute sodium chloride (NaCl) and sodium sulfate (Na_2SO_4) toxicity tests in a low hardness water (40 mg/L as $CaCO_3$) with up to 7 freshwater species: cladoceran (*Ceriodaphnia dubia*), midge (*Chironomus dilutus*), amphipod (*Hyalella azteca*), Gray Treefrog (*Hyla versicolor*), 2 mussels (Pink Mucket *Lampsilis abrupta*; Fatmucket *Lampsilis siliquoidea*), and Fathead Minnow (*Pimephales promelas*). Chloride median 50 percent effect concentration (EC50) values ranged from 1,028 to 5,743 mg Cl/L with an order of species sensitivity: *L. abrupta, L. siliquoidea, H. azteca, C. dubia, H. versicolor, P. promelas*, and *C. dilutus*. Sulfate EC50s ranged from 1,057 to 11,531 mg SO_4/L with an order of species sensitivity: *L. abrupta, L. siliquoidea, H. versicolor, P. promelas,* and *C. dilutus*. We also evaluated the influence of water hardness (15, 30, 60, and

120 mg/L as $CaCO_3$) on the toxicity to 3 sensitive species (*L. siliquoidea, H. azteca, C. dubia*) and found that the toxicity of Cl and SO_4 was significantly decreased with increased hardness, except for *C. dubia*. The results can be used to develop or update WQC, WQS, and other environmental guidelines for Cl and SO_4 to protect freshwater organisms in soft waters.

02.13.05 Analysis of Angiotensin Receptor Blockers and PPARγ in Aquatic Species Via Non-Target Analysis and In Vitro Bioassay Testing

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Lack of knowledge about different chemical in diverse water systems gives researchers limited knowledge of which contaminants are of public health concern. To address this, we are employing 'non-targeted' high-resolution mass spectrometry (MS) followed by a custom annotation pipeline to generate chemical fingerprints in collected water samples to determine whether toxicity data are (or not) readily available for these chemicals in open-source federal databases (i.e. ToxCast). The goal is to find an understudied chemical represented in environmental samples. Water samples were collected from surface waters and wells in Haiti (n=13), North Carolina (n=13), and Sri Lanka (n=11), and chemicals present in these samples were identified by our MS and analysis pipeline. Initial profiling identified 111 chemicals unique to Haiti, 96 chemicals unique to SL, and 256 chemicals unique to NC with 11 chemicals shared by all. Majority of the contaminants had no representation in the ToxCast. The chemical profiles of each region had some overlap (Haiti-SL shared 14 chemicals exclusively, Haiti-NC shared 20, and SL-NC shared 23). One contaminant of concern is pharmaceuticals which made up of 12% for Haiti, 31% for SL, and 16% for NC. An interesting pharmaceutical group to monitor is Angiotensin Type 1 Receptor Antagonist (ARB) used to control blood pressure. A minority of ARBs were found in ToxCast with 3-6% of the tested assays, such as PPARγ, evaluated as suitable for toxicity testing. These results showcase the utility of non-target chemistry to identify chemical fingerprints in complex water samples where limited background information is available. To begin to understand the toxicity of certain ARBs, we performed bioactivity assays for irbesartan and rosiglitazone (a positive agonist for PPARγ) by using transactivation PPARγ reporter assays for human and zebrafish. For irbesartan, results showed AC50 values of 11 µM and 13 µM for human and zebrafish, respectively. For rosiglitazone, results showed AC50 values of 0.2 µM and 2.3 µM for human and zebrafish, respectively. The next steps would be to test the chemicals in an *in vivo* zebrafish embryo model measuring endpoints such as adipogenesis, energy metabolism, heart rate, and morphology. These efforts should lead to a better understanding of exposure and health effects of ARBs to fish in aquatic environments.

02.13.07 Applying Benthic Assessment Tools to Evaluate Ecological Impacts of Seasonal Hypoxia Stress on Benthic Habitat Condition in Pensacola Bay, Florida (USA)

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Seasonal hypoxia (DO < 2 mg L^{-1}) is among the leading stressors affecting estuaries and coastal ecosystems. Assessment tools that integrate habitat condition over biologically meaningful time periods are valuable to resource managers. Thus, the objectives of this study were to evaluate the application of the US M-AMBI (multivariate AZTI marine biotic index, derived from macrobenthic community measures) and the BHQ (Benthic Habitat Quality index, derived from sediment profile images), in Pensacola Bay (Florida), an estuary along the northern Gulf of Mexico coast, to evaluate impacts of intermittent hypoxia on benthic habitat condition. A transect comprised of eight stations was established across the salinity gradient between the Escambia River to the Gulf. Stations were sampled monthly from May to November in 2016 and 2017 for water quality, sediment, and benthic invertebrates. We observed near-bottom hypoxia at every station at least once between June and August, with the most persistent hypoxia observed near the middle of the estuarine salinity gradient. Substrate in the estuary transitioned from 100% sand to ~75% silt at the middle estuary stations and 100% sand at the station nearest the Gulf. Index scores trended toward better habitat condition with increasing salinity, with the lowest scores observed in the middle of the estuary. However, sensitivity of the US M-AMBI was not adequate for separating sites that experienced intermittent hypoxia. The results of this study are a step toward validating the use of these assessment tools to quantify effect of water quality on benthos in a Gulf coast estuary.

02.13.08 Are You Sure You Want to Eat That? Investigating Polychlorinated Biphenyl Concentrations in Lake Erie Fishes

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Lake Erie serves as an important commercial and recreational fishery for residents of Pennsylvania, yet consumption advisories for polychlorinated biphenyls exist for many commonly caught and consumed game species. With consumption limits set at one meal per month due to excessive polychlorinated biphenyl (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. Extractions of PCBs from filets of walleye, freshwater drum, yellow perch, bluegill, brown bullhead catfish, rock bass, white bass, steelhead, and round goby are currently underway. With tentative links to oncogenesis in humans, the PCB concentrations documented within fish filets will be used in cellular assays to better understand the role of dietary exposure of PCBs in effects on human health. The PCB concentrations determined to be present within filets of common recreational fish species will provided 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.

02.13.10 Assessing Impacts of Industrial Effluent on Nutrient Cycling and Food Web Length in a Coastal Marine Ecosystem Using Compound Specific Isotope Analysis

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Industrial pulp and paper mills discharge nutrient-rich effluent wastewater into receiving aquatic environments which can negatively impact aquatic biota. Naturally occurring stable isotopes of nitrogen ($\delta^{15}N$) are an effective tool to establish exposure of aquatic biota to effluent by measuring incorporation of nutrients into organism tissues. Boat Harbour, Nova Scotia, known as one of the most contaminated sites in Canada, was formerly a wastewater treatment facility for pulp mill effluent with subsequent discharge from the facility into a coastal marine environment. Effluent release into Boat Harbour ceased in 2020 and remediation of the ecosystem is projected to begin in 2021. To assess ecosystem impacts and establish baselines to evaluate effectiveness of remediation activities on the aquatic system, an ecosystem-wide approach to determining spatial impacts of industrial effluent on the aquatic food web of the coastal marine environment was required. This research aimed to investigate the sources of bioavailable nitrogen along a pollution gradient to characterize impacts of historical effluent on coastal food webs and determine if exposure to pulp mill effluent alters the length of coastal food webs. Compound specific nitrogen isotope analysis of amino acids was used as a methodological tool to trace energy flow and calculate the trophic position of organisms inhabiting the coastal ecosystem, such as macroalgae, mussels, lobsters and native fish. This study assisted in quantitatively characterizing the biogeochemistry and trophic ecology of an ecosystem impacted by pulp mill effluent prior to remediation and assessed the impacts of effluent on coastal food web structure.

02.13.11 Assessing the Impacts of a Common Deicing Agent on the Viability, Embryonic Development, and Behavior of the Freshwater Gastropod Physa acuta

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In 2014, nearly 24.5 million tons of road salt were distributed on public roadways in the United States. The most common compound, NaCl, accounts for 90% of all applied deicing solutions. Over half of this volume has been shown to directly enter waterways as runoff, resulting in increased salinity conditions in both freshwater and brackish environments. While the USEPA recommends that concentrations do not exceed a 4d average of 230mg/L more than once every 3 years, recent studies have observed concentrations of over 600mg/L during winter months in some US locations. NaCl is known to inhibit algal growth, reduce respiration rates of bacteria in activated sludge, and increase mortality in all life stages of multiple amphibian species. However, the effects of NaCl on aquatic invertebrates has been relatively unexplored. The freshwater gastropod *Physa acuta* is well-suited due to its wide geographic presence, hermaphroditic reproductive life cycle, and rapid development. Thus, the goals of this study were to determine the effects of aqueous NaCl exposure on 1) *P. acuta* egg cluster viability and development and 2) adult mobility behavior (average mobile speed, average speed, total distance traveled, acceleration, number of frozen events and time spent frozen). Newly laid egg clusters were collected and exposed to 0, 100, 250, or 500mg/L NaCl using a static exposure assay. Viability and developmental stage of each embryo was assessed every 24hrs until all eggs were either inviable or had hatched. Adults were exposed to 0, 100, 250, 500, and 1000mg/L NaCl for 7d using a static replacement assay. Individual mobility was recorded and quantified on 0, 3, and 7d using ToxTrac (v. 2.83). While this experiment is still ongoing, we expect to find 1) a dose-dependent increase in cluster mortality for both embryos and adults as well as developmental delays and 2) decreases in speed, total distance traveled, and acceleration as well as increases in frozen events and the time spent frozen. As there is currently no research on the effects of NaCl on *P. acuta*, these findings provide novel insight into the impacts of a commonly utilized deicing agent on a non-model freshwater invertebrate species, suggesting that similar effects may also be exerted on endangered or keystone aquatic invertebrate species.

02.13.12 Assessment of the Toxic, Neurotoxic and Genotoxic Effects of Sediments in Environments Associated with the Papaloapan River, Veracruz, Mexico

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Sediments are a very important components in aquatic ecosystems because they play an important role in the exchange of chemical substances between the particulate, dissolved and biological phases. Sediments generally act as natural adsorbents, capturing a wide variety of compounds not only nutrients but also toxic compounds. The objective of this study was to carry out an evaluation of the toxic, neurotoxic and genotoxic effects of sediments obtained in systems associated with the Papaloapan River Ver. The study area of the present work is located between the extreme coordinates 18 ° 18 ‘ to 18 ° 47’ North and 95 ° 44 ‘ to 95 ° 51’ West, that includes the river environments: Chacaltianguis and Dos Bocas; lagoon: El Pájaro and El Embarcadero and coastline: Playa de Alvarado. 11 sediment samples were collected in June (dry season) and October (rainy season). The following parameters were evaluated from the samples: pH, volatile sulfides, ammonium, organic matter, conductivity and texture. Sediment toxicity was determined through a battery of bioassays using *Daphnia magna*, *Moina macrocopa* and the microalgae *Pseudokirchneriella subcapitata* as test organisms. The neurotoxic effect was determined with an *in vitro* test and the genotoxic effect with the SOS-Chromotest test. The information generated was integrated into a multivariate analysis to establish the degree of contamination of the sediments. The results obtained showed that the mortality percentages obtained in the toxicity bioassays ranged from 20 to 50%, which means that the contamination degree of the sediments was moderately to highly contaminated. The mortality percentages obtained in the samples collected in the Embarcadero Lagoon, and Laguna de los Pajaros were 50%, which indicates that the samples are highly contaminated in these sites also presented compounds with genotoxic and neurotoxic effects. A small population of fishermen and a remnant of gas emanation near oil fields are located in these sites. Carrying out activities such as growing organisms, fishing or recreational activities in these places could pose a health risk.

02.13.13 Biomarkers As Indicators of the Health State of Pteria Sterna in Ensenada DE LA Paz, B.C.S. Mexico

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The urbanization process of Ensenada de La Paz has increased the contribution of xenobiotics in the coastal zone, causing an increase in the levels of xenobiotics that together with the climatic changes, constitute a risk for the health status of the pearl oyster *Pteria sterna*, resource of great economic importance in this area. In this work an evaluation of the effects of the contaminants present in the cultivation sites, in the health status of *P. sterna* was made by means of the evaluation of biomarkers. Organisms were collected in two locations of Ensenada de la Paz, a site in front of Isla Gaviota (24º17'21.75''N and 110º20'26.9''W) and the other site in the Marina of La Paz (24º09'09.77 '' N and 110º19'25.75 "W). 20 organisms were collected from each site during 2 seasons (summer and winter). The condition index was evaluated. The degree of lipoperoxidation (TBARS), the activity of antioxidant enzymes: SOD, CAT, GPx, AChE activity and MN frequency were also determined. The results obtained in the condition index, lipoperoxidation, CAT, AChE and MN in the two sites showed a significant difference ($p < 0.05$). The organisms with the highest growth rate were the collected in Isla gaviota. A higher degree of lipoperoxidation was observed in the gills of molluscs collected in the marina. The activity of CAT was greater in these mollusks. AChE activity was lower in pearl oysters of the Marina. The frequency of MN was higher in the pearl oysters of the Marina. The data obtained in the evaluation of lipoperoxidation, AChE, SOD, CAT and MN in *P. sterna* of La Marina could be related to the presence of metals that cause negative effects on their health status.

02.13.15 Characterization and Interpolation of PCB and PBDE Levels in Sediment in and Around Resident Killer Whale Critical Habitat Along the Coast of British Columbia, Canada

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British Columbia's (BC) Northern and Southern Resident Killer Whale (*Orcinus orca*) populations are listed as threatened and endangered in Canada, respectively, and persistent bioaccumulating contaminants, such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) pose a key threat to their survival and recovery. PCB and PBDE concentrations in subtidal surface sediment collected from 97 sites along the BC coast as part of Ocean Wise Conservation Association's *PollutionTracker* Program, Fisheries and Oceans Canada's Whale Contaminant Program and Environment and Climate Change Canada's Disposal at Sea Program were analyzed to assess resident killer whale habitat quality. Geostatistical and principal component analyses (PCA) were used to identify distributions and patterns of PCBs and PBDEs in and around resident killer whale critical habitat and to compare levels in sediment to Canadian environmental sediment quality guidelines (SQGs). Total PCB and PBDE concentrations and congener and homolog profiles in sediment varied among sites. Congener profiles as characterized by PCA showed more dispersion among sites for PCBs than PBDEs (PCBs: PC1 (38.4%) and PC2 (11.9%); PBDEs: PC1 (28.4%) and PC2 (14.5%)) and were correlated with octanol-water partition coefficient (LogKow; $p < 0.002$) or concentration weighted average of octanol-water partition coefficient (CWALogKow; $p < 0.001$) to PC1. Total PCB and PBDE concentrations exceeded BC interim sediment quality guidelines (PCBs: 0.0037 ng/g dw; PBDEs: 1 ng/g dw), developed to be protective of killer whales, at all sites and at approximately 35% of the sites by 4 (PCBs) and 2 (PBDEs) orders of magnitude, respectively. The probability of exceeding these guidelines for most of the interpolated areas over SRKW critical habitat was > 0.5, suggesting that the SRKW population is at risk from current levels of both PCBs and PBDEs along the BC coast. These results provide the most comprehensive assessment of PCBs and PBDEs in British Columbia waters and provide guidance that can be used for source control that will improve the quality of resident killer whale habitat.

02.13.16 Characterizing Contaminant Concentrations in Priority Chinook Salmon Stocks Consumed by Resident Killer Whales in the Northeastern Pacific Ocean

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The critically endangered, transboundary Southern Resident Killer Whale (SRKW) (*Orcinus* orca) population faces significant threats including reduced abundance of their primary prey (Chinook salmon, *Oncorhynchus tshawytscha*), physical and acoustic disturbance, and high levels of endocrine disrupting contaminants. However, the sympatric Northern Resident Killer Whales (NRKW) that also primarily consume Chinook salmon continue to increase in population and have lower contaminant burdens. Studies have reported adverse health effects from contaminant burdens in transient killer whales and NRKWs, and contaminant exposure modeling has predicted protracted health risks for both resident killer whale populations. Despite Chinook salmon from the Fraser River watershed in British Columbia, Canada compromising up to 90% of the SRKW diet during the summer months, little information exists on contaminants in these priority stocks, as well as other priority Chinook stocks from other Canadian rivers. Characterizing contaminant concentrations in SRKWs is exceedingly difficult due to their small population size, endangered status, and long-range habitat movements. Chinook salmon can be used as a proxy for helping to characterize contaminants and their risks to SRKWs. In the current study, muscle tissue from nine priority Chinook stocks consumed by SRKWs and NRKWs were assessed for concentrations of five priority contaminants classes (PCBs, PBDEs, OCPs, Dioxin Furans, and Chlorinated paraffins) and their profiles, as well as stable isotope ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) profiles. This enabled a characterization of contaminants in priority Chinook stocks and allowed for a preliminary assessment of exposure between two sympatric resident killer whale populations. Collections were done via partnerships with First Nations, recreational and commercial anglers, and the Albion test fishery. Stable isotopes and stock migrations were used to investigate variables affecting differences in the accumulation of contaminants. This evaluation of contaminants found in resident killer whale priority Chinook stocks will help to deliver refined guidance to support the wider conservation agenda for these at risk species.

02.13.17 Copper Accumulation Through Trophic vs. Direct Water Exposure Routes in a Dragonfly Nymph/Mosquito Larvae Laboratory Food Chain

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Aquatic organisms can uptake metal contaminants directly from contaminated water or by consuming contaminated prey. Evaluating the relative importance of these routes is a critical step toward understanding the movement of metals into and through aquatic food webs. An essential metal such as Copper (Cu) can become toxic to an organism if accumulated to excessive concentrations. Previous work established that the aquatic dragonfly nymph *Erythemis simplicicollis* accumulates elevated concentrations of Cu when living in contaminated wetlands. However, the route of uptake of accumulated copper is not well understood. We evaluated copper accumulation within a two-trophic level laboratory food chain using *E. simplicicollis* dragonfly nymphs and *Aedes aegypti* mosquito larvae to gain better insight. Treatments consisted of nymphs exposed to copper through diet (*A. aegypti* larvae prey exposed to 100 ppb Cu contaminated water), water (100 ppb Cu), diet + water, and a control. Each treatment included 15 replicates, and exposures lasted 32 days or until nymph death. We determined whole body Cu concentrations for each nymph and confirmed contaminant exposure by analyzing copper concentrations of contaminated water and mosquito prey. We further evaluated the potential impacts of contaminant exposure to the nymphs by comparing nymph mortality, growth, number of molts, and feeding rate among treatments. The highest copper concentrations accumulated in *E. simplicicollis* when exposed to contaminated water, both in the water only and in the diet + water treatments. Overall, mortality was greater when exposed to copper contaminated water. In this simple food chain, exposure to copper through water, rather than trophic exposure, significantly influenced metal accumulation in this predatory dragonfly nymph.

02.13.18 Daphnia magna Model to Understand Lipid Metabolism

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Background: Several factors including the environment, exogenous and endogenous compounds can alter lipids storage, accumulation, and metabolism in organisms. Lipids can also be additional energy stores that give living organisms a substantial metabolic benefit, however, an energy distortion could lead to lipid diseases and liver dysfunctions. Overall growth and development are seemingly affected in crustaceans. *Daphnia magna* specifically has its growth, molting, and reproductive functions been regulated by the amount and fate of storage lipids. Tributyltin (TBT), one of the well-known toxic pollutants to be found in the aquatic environment, is a substance that resists degradation and is absorbed by organic materials such as bacteria and algae.Objective: With little knowledge about the formation and metabolism of lipid droplets in *Daphnia manga* both physically and transcriptionally, we employed this *in vivo* model in our study. Hence, in this study, we seek to develop a model that can be used to study the mechanisms involved in lipid metabolism associated with accumulation, uptake, and regulation.Methods: *Daphnia magna* individuals of 5 days old were exposed to TBT 0, 1.0, and 1.0 µg/L for 48 h. Then, Oil Red O and Nile red staining processes were carried out, stained individuals were observed under a stereomicroscope and confocal microscope, respectively. The transcription of selected genes (*rxr, cer2, hr96, magro, man, NPC1b* & *SM3*) involved in fat metabolism was also analyzed using qPCR techniques.Results and Conclusions: Microscopic *Daphnia magna* images from both Nile red and Oil red staining showed analogous patterns from our findings. At the transcription level, *rxr, cer2* and *SM3* had been significantly altered. The *Daphnia magna* successfully responded to our tests and significant effects were observed as expected. Our findings indicate that TBT facilitates the uptake and accumulation of lipids in *Daphnia magna*. Therefore, the *Daphnia magna* model presents an endless potential to tap into and can be an alternative to test lipid metabolism altering chemicals. Nonetheless, further research needs to be done on lipid metabolism mechanobiology, to understand how cells respond to mechanical signals that cause a disruption in lipids production and use.

02.13.19 Data Generation to Support Optimization of a Feasible Feeding Methodology for the Pimephales promelas 7-Day Growth Test

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The fathead minnow, *Pimephales promelas*, chronic toxicity test is commonly employed in research and regulatory testing laboratories. In the test, a sublethal 7-day biomass endpoint is generated to estimate toxicity; growth (e.g. final mass – initial mass) of the larvae is evaluated. Live *Artemia* nauplii is the standard diet in this freshwater test, but they are marine organisms and perish rapidly. Implementation of a specific *Artemia* feeding ration is critical to method accuracy, consistency, and precision. The use of non-prescriptive terms and protocols in published guidelines and methodologies, specifically related to "*ad libitum"* feeding, allows laboratories to interpret meaning and may lead to procedural execution errors and variability between individual bioassays and laboratories. This impacts the consistency and statistical power of toxicological test and thus, the sublethal growth endpoints generated for regulatory decisions. The current study sought to determine the ideal combination of three variables, 1) fish density, 2) feeding ration, and 3) feeding frequency, that would allow for optimal *P. promelas* growth, while also fostering clear, prescriptive guidance for the future conduct of the chronic toxicity test assay. Analysis of the data indicated statistically significant evidence that treatments receiving food three times daily (in 6-hour intervals) with the highest ration, regardless of fish density, produced fish with the highest masses ($p < 0.05$). Current lab feeding methods are understandably coordinated around business hours, but larvae mass was greatest when a third feeding was supplied outside the typical eight-hour business day. While it was still possible to produce fish with statistically significant high masses ($p < 0.05$) with two feedings within the business day, the cost benefit of a prescriptive feeding ration supplied at wider intervals should be considered.

02.13.20 Detoxification Pathways of Lampricides in the Tissues of Sea Lamprey (Petromyzon marinus) and Non-Target Fishes

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The applications of lampricides, lamprey specific pesticides, in the control of sea lamprey (*Petromyzon marinus*) populations are the main component of the Great Lake Fishery Commission and the sea lamprey control program (SLCP). However, even 70 years since their discovery, much is unknown about their metabolism and modes of action. The TFM (3-trifluoromethyl-4-nitrophenol) was chosen based on its specificity to target sea lamprey with minimal effects on other fishes. The applications of TFM are often paired with niclosamide (2′,5-dichloro-4′-nitrosalicylanilide; NIC) as their combination increases the toxicity of TFM. Niclosamide seems to act similarly to TFM; however, its effects are much broader, resulting in a higher cost to non-target fishes. The selectivity of TFM to only target sea lamprey comes from lamprey's reduced ability to detoxify the parent compound (TFM) into much less toxic and easier to excrete metabolites TFM-Glucuronide and TFM-Sulfate. These metabolites are the markers of TFM metabolism and are used to quantify the rate of TFM detoxication in sea lamprey and non-target fishes. We report the development of 96 well vacuum manifold solid-phase extraction (SPE) method for tissue extractions. As well as liquid chromatography-mass spectroscopy (LC-MSMS) protocol to measure the parent TFM and NIC compounds in the liver and muscle tissues of larval sea lamprey and non-target fishes. Due to commercial unavailability, we also present the protocol for TFM metabolites synthesis, which, once purified and quantified, are used as standards for LC-MSMS. The mentioned methods allow direct testing of the lampricides metabolic rates and pathways in tissues of fishes exposed to various concentrations and combinations. Preliminary results provide evidence of TFM and NIC accumulation in sea lamprey livers and muscles. At the same time, in non-target fishes, there is a time-dependent reduction in the levels of TFM in both liver and muscle tissues. This research provides insight into the modes of actions of the lampricides that can lead to more environmentally sustainable solutions of sea lamprey population controls.

02.13.21 Does Sample Location or Collection Date Impact Species Distribution or Sensitivity of Hexagenia Spp?

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Two mayfly species *Hexagenia limbata* and *Hexagenia rigida* nymphs are commonly used in toxicity bioassays. Although the nymphs can be raised in the laboratory, due to their unique life history, eggs must be collected and stored annually. *Hexagenia* are collected from various locations and at varying times during their emergence. Since the two species commonly used in toxicology may have different sensitivities, it may be important to identify locations and times of emergence when the desired species is most available. Additionally, understanding population variation particularly among sampling locations and collection times, may also explain variation among toxicological studies. The current study collected *Hexagenia* from three sampling locations at three times during the emerging season. At each collection, adults were collected and identified to determine species and sex ratios. Additionally a subsample of eggs were collected and hatched to examine differences in hatch rates, growth rates, and sensitivity to NaCl (a representative pollutant). Understanding these patterns of natural variation will help to understand variation in these species that may be observed in toxicological studies.

02.13.22 Ecotoxicity and Accumulation of Per- and Polyfluoroalkyl Substances in the Fathead Minnow

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Per- and polyfluoroalkyl substances (PFAS) have emerged as contaminants of environmental concern following release from industrial practices and widespread use of Aqueous Film Forming Foam (AFFF). Previous studies have shown that surface water contamination with PFAS is very common and likely to be the dominant exposure media for wildlife. To date, four fathead minnow PFAS toxicity studies have been conducted, those include exposures to PFOS, PFHxS, PFNA and the mixture of PFOS and PFHxS. The study design is the same among the experiments, where adult fish are exposed over the reproductive life-stage for 42 days and offspring collected at a mid-point are exposed for 21 days of development. The exposure is a static-renewal where 50% of the water is exchanged three times per week. Of these studies completed, PFOS has been shown to result in greater toxicity at lower concentrations compared to all other PFAS tested. The no observable effect concentration (NOEC) for PFOS was 44 µg/ L and the lowest observable effect concentration (LOEC) was 88 µg/L based on reduced growth in juvenile (F1) fish. Compared to the NOEC for PFHxS of 1200 µg/L, the highest concentration tested. Additionally, PFAS appears to accumulate in brain and gonad tissue of the fish; however, PFOS concentrations are comparatively greater than PFHxS in both tissues. Some similarities in results of the PFOS-PFHxS mixture and the PFOS alone studies are apparent. For example, both report apical effects occurring in the juvenile life-stage and this same endpoint is impacted in the PFNA exposures at concentrations ≥ 250 µg/L. Importantly, follow-up testing using larval fish exposures from clean (non-PFAS exposed) parental fish do not result in toxicity at these concentrations and therefore, we believe there is likely maternal transfer contributing to developmental toxicity. This is currently being further explored through a full life-cycle fathead minnow study that underway.

02.13.23 Effects of Dietary Selenium on the Freshwater Amphipod, Hyalella azteca

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The US Environmental Protection Agency (USEPA) established water quality criteria for selenium in 2016. Their data review concluded that fishes were the most sensitive group of aquatic organisms to chronic toxic effects of selenium (Se) based on effects on reproduction and early life stages of numerous fish species. Chronic Se toxicity data available for freshwater invertebrates indicated that invertebrates were less sensitive than fish. However, the number of suitable toxicity studies with invertebrates was limited (3 species) and not all these studies included reproductive endpoints. The USEPA is concerned the available invertebrate toxicity data may not adequately protect aquatic biota from chronic Se toxicity especially in fishless water bodies. To provide additional information on the effects of Se on invertebrates, we conducted a 42-day chronic full life cycle exposure. Prior to the definitive study, an 11-day feeding study was conducted to evaluate the suitability of feeding selenized yeast, diatoms, and flake food, alone and in combination, to the amphipod *Hyalella azteca* and the midge *Chironimus dilutus.* This work determined the diet of a mixture of selenized yeast and diatoms provided adequate nutrition. Rangefinding 28-d exposures were then conducted with *H. azteca* and *C. dilutus*, which indicated that *H. azteca* was more sensitive to Se exposure (growth and survival). Survival of *H. azteca* was affected between 12.5 µg/g and 50 µg/g Se in the diet, and exposure was confirmed through determination of Se tissue concentration. A full 42-day lifecycle experiment was conducted in 2021 with *H. azteca* and toxicity endpoints measured in the study included survival, growth, and reproduction (young/female). Preliminary results indicate survival was affected between 50 and 100 µg/g Se in the diet. Growth and reproduction data are currently being analyzed but indicate effects at less than 12.5 µg/g Se in the diet.

02.13.24 Examining the Practice of Pre-Washing Nets as a Method of Mitigating Toxicity From Insecticide Treated Net Fishing

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The distribution of insecticide treated mosquito nets (ITNs) is a great public health success story, with studies suggesting these nets have 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 purposes, including fishing. Coinciding with incidences of off use of ITNs for fishing, 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. However, it is unclear whether this leaching persists beyond the initial submersion of the net. The purpose of our study is to determine whether prewashing the nets reduces leaching and toxicity associated with ITN fishing. We conducted a series of exposure experiments using *D. magna* by introducing them to different sized ITNs. These nets ranged from $1cm^2$-$20cm^2$. The nets were pre-washed once, twice, or three times before being used in exposure experiments. Each washing consisted of submerging the net for 30 minutes in 300mL of moderately hard water and dried for 30 minutes prior to exposure. Once the nets were placed in exposure beakers, they remained for 30 minutes and were then removed and discarded. After exposure we measured growth and mortality end points in the organisms. Additionally, water samples were analyzed by GC-ECD to determine leaching concentrations of pyrethroids. Results from pre-washing experiments showed a reduction in toxicity when the nets were prewashed for 30 minutes, when compared to non-prewashed nets from previous experiments. Results from the leaching experiments will be presented during the meeting as well. Results from these studies indicate that the use of ITNs for fishing represents a significant potential hazard to aquatic organisms and that leaching from the nets is occurring. However, we have found that prewashing the nets may be useful to reduce aquatic organism toxicity. Educational strategies may need implementation alongside ITN distribution to decrease this practice and reduce risk to aquatic organisms.

02.13.26 Flow-Through Passive Dosing of 1-Methylnaphthalene to Determine the Effect of Dynamic Exposures on American Lobster (Homarus americanus) Larvae

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Crude oil is a complex mixture made up of thousands of different chemical components. To better understand the toxicity of crude oil, mathematical models rely on individual polycyclic aromatic compound (PAC) toxicity data to predict and assess the impact of crude oil spill. Crude oil exposures in the environment are often dynamic in nature and are more likely to occur over the period of minutes to hours as opposed to 48 - 96 hour exposure used for the majority acute toxicity tests. There is limited data available to date on the impact of brief exposures to PACs despite their importance in oil spill models. In this study we conducted dynamic exposures of Stage I American lobster (*Homarus americanus*) larvae to 1-methylnaphthalene (1-MN) to estimate the uptake and elimination rate of PACs in a dynamic exposure system. The exposures were conducted in glass flow through vessels, with flow rates of 5, 3, and 1 mL per minute. Each trial included 4 vessels: 3 1-MN replicates, and 1 seawater control vessel. Exposure concentrations were measured in the vessel every 10 minutes and immobilization and mortality were scored every 5 minutes. Trials were terminated once all the 1-MN vessels reached 100% mortality. Passive dosing with PDMS O-rings was used to both spike the seawater with 1-MN and maintain the exposure concentration of the PAC stock used. O-rings were added to prepared 1-MN methanol stock solutions and were shaken at 200rpm to equilibrate for 72 hours. The loaded O-rings were then rinsed with deionized water and added to seawater to equilibrate for 24 hours. The exposure concentrations were determined using GC-MS validated fluorometry measurements throughout the trial. The 5 mL, 3 mL, and 1 mL per minute flow rates had LT50s of 109.8 ± 0.4, 160.3 ± 7.6, and 417.8 ± 8.5 minutes, respectively. The exposure dose that resulted in 50% mortality, as determined by the area under the concentration over time curve, was very similar for the 5 and 3 mL per minute flow rates (79.7 ± 3.3 and 70.1 ± 1.1; respectively). The exposure dose for the 1 mL per minute exposure was much larger (137.4 ± 4.5) suggesting the larvae were able to eliminate the PAC more effectively during these more gradual increases in exposure concentrations. The data collected in the study will be used to both improve oil spill models and improve our understanding of the toxicokinetics of PACs.

02.13.27 Influence of Water Hardness on Chronic Toxicity of Potassium Chloride to a Freshwater Mussel (Lampsilis siliquoidea)

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Elevated concentrations of potassium are often measured in effluents from a variety of anthropogenic sources, including industrial and municipal wastewater treatment plants, oil and gas production operations, and mineral extraction processes. Recent studies have demonstrated that freshwater mussels are highly sensitive to K in acute and chronic exposures; however, the acute K toxicity to mussels decreased with increasing water hardness. The potential influence of water hardness on chronic K toxicity to mussels and other freshwater organisms has not been studied in detail. This study aimed to evaluate the chronic toxicity of potassium chloride (KCl) to a commonly tested unionid mussel (fatmucket, *Lampsilis siliquoidea*) in waters with a wide range of hardness (25 to 300 mg/L $CaCO_3$). Chronic 28-day tests were performed according to ASTM standard test methods and conducted simultaneously in five hardness waters: a well water with a hardness of 300 mg/L as $CaCO_3$ and four waters prepared by diluting the well water with deionized water to a hardness of 25, 50, 100, or 200 mg/L. This hardness range is representative of most waters of the US. Five flow-through diluters were used; each diluter delivered five K concentrations with a 50% dilution series plus a control (one of the five hardness waters). Mussels were fed an algal mixture automatically every four hours with each cycle of water delivered by the diluters. At the end of the tests, surviving mussels were counted and preserved for subsequent length and dry weight measurements. Preliminary results based on survival (growth measurements are ongoing) indicated that 20% lethal concentrations (LC20s) ranged from 14 to 50 mg K/L in the different hardness waters. There was a strong linear correlation between LC20s and hardness (r^2=0.98) and LC20s increased significantly with increasing hardness (p< 0.001). The results of this study will be useful for establishment of chronic K toxicity thresholds across a broad range of water hardness in the US and can be used to develop or update environmental guideline values for K to protect freshwater organisms.

02.13.28 Iodide Rescues Thyroid Disruption on a Zebrafish Vertebrate Model

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The inherent advantages of the zebrafish (*Danio rerio*) vertebrate model makes it a commonly used alternative to *in vitro* and *in vivo* models in assessing both human and aquatic toxicity. These advantages include their homology with mammals, small size and rapid development, and the cost-efficiency of the model. The use of chemicals that can disrupt the endocrine system can result in adverse effects on reproductive, developmental, neurological, and/or immune functions in both humans and wildlife and there is an increasing concern about these environmental impacts. Iodide is crucial for thyroid hormone synthesis and function and can decrease the response of the thyroid gland to the thyroid-stimulating hormone, suggesting a major role in the negative feedback loop of this gland. This work aimed to investigate the potential role of iodide exposure in modulating the impact of endocrine-disrupting chemicals (EDCs). For this purpose, Sodium Iodide was co-exposed with different concentrations of two known thyroid disrupting (TD) substances such as potassium perchlorate, and propylthiouracil. Firstly, the transgenic line tg(tg:mcherry), which expresses red fluorescence in the thyroid gland under the thyroglobulin promoter, was used to evaluate TD chemicals. The quantification of this fluorescence intensity allowed monitoring *in vivo* the upregulation of the thyroglobulin gene expression as a compensatory reaction to thyroid gland disruption. Secondly, the gene expression of thyroid-related genes (*tsh*β, *tg*, and *tpo*) was studied. Finally, thyroid hormone levels (T4, T3, 3,5-T2, and 3,3'-T2) were quantified by liquid chromatography (LC/HRMS) in whole-embryo extracts. Our data show that single exposure of both chemical compounds induced an increase of the fluorescence of thyroglobulin in the tg(tg:mcherry) transgenic line, overexpression of thyroid-related genes, and a concomitant decrease of T4 hormone levels. Preliminary data show that iodide co-exposure could reverse the TD effect of potassium perchlorate but not that of propylthiouracil. Zebrafish offer a sensitive and cost-effective model to screen and evaluate potential EDCs.

02.13.29 Kynurenine to Tryptophan Ratio: A Biomarker for Stress in Rainbow Trout (Oncorhynchus mykiss)

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Tryptophan (Trp) metabolism down the kynurenine pathway (KP) in particular plays a crucial and not completely elucidated role in the regulation of the immune response during stress. The first step of the KP is controlled by two rate-limiting enzymes, namely: tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO). The [Kyn]/[Trp] ratio (KTR) is used to reflect the activity of IDO and is used as an alternative to the measurement of IDO activity since it is often too low for detection even under enhanced immune activation. This study aims to validate Kynurenine (Kyn) and Trp measurements in fish liver tissue to assess its usefulness as a diagnostic biomarker for acute stress. The method was based on isotope dilution high-performance liquid chromatography positive ion electrospray tandem mass spectrometry method to separate and detect Trp and Kyn in fish liver and brain. Specific multiple reaction monitoring transitions were employed for each analyte to ensure method selectivity. Rainbow trout (*Oncorhynchus mykiss*) liver samples were lyophilized and liquid-solid extracted using cold (4ºC) acetonitrile: water (65: 35, pH 7). The extracts were sonicated in ice water for 12 mins, vortexed for 1 min, and then centrifuged at 14,500 rpm for 15 mins to remove co-extracted biogenic material. The respective method performance characteristics for Kyn and Trp were: linearity: 2.5-750 and 2.5-5000 pg/µL, limits of detection/quantitation: 1.79/5.95 and 1.20/3.99 pg/µL, recoveries ranged from 70 to 102% with intra-day repeatability ≤ 15% RSD. The matrix effects were negligible. Fish held in the laboratory were subjected to 5 minutes of a physical stress event by vigorous net chasing and a hypoxic event. The fish were sacrificed at 4 and 48 hrs post-stress event. The KTR was greater in the fish liver in the 48hr post-stress (n = 10) exposure group relative to controls (n = 10, $p < 0.05$); a similar increase was not observed in fish in the 4hr post-stress exposure group. Hepatic cortisol levels were also elevated in both treatment groups relative to their respective controls. These results suggest that KTR is a promising acute stress biomarker in fish liver. Efforts are ongoing to assess whether the KTR can be used as a biomarker for fish exposed to aquatic contaminants and other environmental pollutants and stressors.

02.13.31 Long-Term Monitoring of Polychlorinated Biphenyls (PCBs) in Fish From Eastern Tennessee

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Environmental contamination due to human activities is a major concern, particularly for persistent chemicals. Within catchments, there is great potential for persistent contaminants to be transported, either through adsorption or biological uptake, with downstream locations acting as sinks for accumulation. Polychlorinated biphenyls (PCBs) are a widespread legacy pollutant from industrial activities that have been linked to numerous negative health outcomes for wildlife and humans. Here we present long-term observations of PCB body burdens within fish found in lower-order tributaries on the Oak Ridge Reservation, an impacted US Department of Energy property in eastern Tennessee, and a large reservoir system adjacent to it composed of parts of the Clinch and Tennessee Rivers. Given that the reservoir system has experienced no direct PCB mitigation activities, this contamination offers an opportunity to explore potential natural attenuation of PCBs within a large lotic ecosystem. Temporal and spatial patterns in PCB concentrations were compared between multiple stream and reservoir sites, with a focus on gamefish species (sunfish and catfish) with potential to directly affect human health. Trends reported in this study are compared to similar efforts from other regions to contextualize and evaluate consistency in responses since the banning of PCB production in 1979.

02.13.33 Meta-Analysis Shows Environmental Contaminants Elevate Glucocorticoid Levels in Fish

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Wild vertebrates are at risk of long-term exposure to environmental contaminants (ECs) due to the persistence and widespread distribution of ECs globally. Long-term exposure to ECs could result in changes to levels of glucocorticoid (GC) hormones in non-target vertebrates. Short-term changes in GC hormones during stressful events are necessary for survival as they aid the organism in maintaining homeostasis during and after the event. However, long-term changes to GC levels are associated with adverse health and/or reproductive outcomes. We conducted a meta-analysis to summarize the relationship between experimental exposure to ECs and fish GC levels, and to explore potential moderator variables that could help explain observed variation in effect sizes. Captive-bred fish exposed to ECs have average baseline GC levels 1.5X higher than unexposed fish. Additionally, GC responses of fish are dependent on the class of EC to which they were exposed; fish exposed to pharmaceuticals have average baseline GC levels 2.5X higher than unexposed fish. Our analyses further suggest that chronic exposure to ECs is negatively correlated to effect size, potentially because of acclimation or HPA-axis exhaustion. Effect sizes did not differ between baseline and challenge-induced GC measures. We also found no difference in baseline GC response after EC exposure between captive-bred and wild-caught fish; studies using captive-bred fish may enable inferences about GC responses to ECs for wild individuals. We recommend that future research focuses on a broader array of EC types and vertebrate classes, on mechanisms of HPA axis effects due to particular ECs, and on the effect of multiple stressors on both baseline and challenge-induced GC levels of vertebrates.

02.13.34 Metabolic Profiling of Daphnia magna Sub-Lethal Exposure to Bisphenols Using Targeted Liquid Chromatography Tandem Mass Spectrometry

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Daphnia magna is a keystone freshwater organism, positioned at an intermediate level in many aquatic food chains, and is frequently used in ecotoxicological studies as a sentinel species for toxicity assessments. Bisphenols are chemicals mostly used in the production of polycarbonate plastics and epoxy resins. Bisphenol A (BPA) is the most used and can be found in can coatings, dental fillings, as additives in thermal papers, among other applications. Due to concerns with BPA acting as an endocrine disruptor to humans and wildlife, alternative chemicals, such as bisphenol F (BPF) and bisphenol S (BPS), have been increasingly implemented in similar manufacturing processes, however, it is unclear if BPF and BPS may have the same impacts on aquatic organisms as BPA. In this study, the *D. magna* metabolome is used to assess the sub-lethal toxicity of the three bisphenols (BPA, BPF, and BPS). In an acute 48-hour test, *D. magna* were exposed to a range of sub-lethal concentrations for all three bisphenols. These concentrations were fractions of the EC_{50} (concentration that induces a response to a toxicant in half of the population) for the respective bisphenols. Targeted metabolite analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to measure 34 different metabolites, which are linked to various biochemical pathways. Principal component analysis (PCA) and partial least square-discriminant analysis (PLS-DA) were used to evaluate changes in the metabolite profile relative to the control (unexposed group). Overall, BPA exposure groups were the most distinct relative to the control treatment ($p \leq 0.05$) in comparison with BPF and BPS. Metabolite changes were used in pathway analysis to ascertain which metabolic pathways were perturbed with sub-lethal exposure to the bisphenols. All bisphenols shared some perturbated pathways, such as aminoacyl-tRNA biosynthesis and arginine and proline metabolism, suggesting a common compound class result. Additionally, each contaminant also led to unique changes, indicating different toxic responses that are specific to different bisphenols. This study was able to demonstrate that all different bisphenols can alter the metabolism of *D. magna* at sub-lethal levels. The observed alterations indicate that these chemicals can exert similar impacts on *Daphnia* health while they had also perturbated the metabolism in a unique fashion.

02.13.36 Microplastic Contamination Across an Urban Gradient in the Salish Sea, WA

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Global anthropogenic production and pollution have grown substantially over the last 70 years, and with it, come the projected exponential rise of microparticle and microplastic pollution. In coastal environments, suspension-feeding bivalves have historically served as bioindicators to detect pollution (e.g. Mussel Watch). Mussels are known to sequester pollution and toxics from surrounding water, making it relatively easy to analyze water contamination levels through mussel tissue samples. The Salish Sea extends past the US-Canada border, contains the Port of Seattle and is adjacent to several large cities including Tacoma, Seattle, Victoria, and Vancouver BC. Numerous studies demonstrate that mussels ingest microplastic in the wild, with the overarching finding that mussels in more urbanized environments contain higher concentrations, however, the local extent of ingestion and contamination is unknown. Through analyzing mussel tissue, we can better measure and understand microplastic contamination levels biotically available to organisms on a spatial scale. In this study, we compare mussel microplastic contamination across 15 sites in the Salish Sea, ranging from highly urbanized areas (Tacoma and Seattle) to rural areas (Tatoosh Island). Our hypotheses are twofold: 1) mussel microparticle contamination increases along an urban gradient and 2) microparticle morphotype composition ingested by mussels is site dependent. We chemically digested mussel tissue with a standardized wet oxidation extraction protocol adjusted to the weight of mussel tissue, visually counted particles through a microscope, and chemically identified polymer types with Raman Spectroscopy. We found mussel contamination ranged 0-6 microparticles per gram of mussel tissue, higher than previous bivalve studies. A difference in microparticles contamination as well as morphology and color between sites was observed, however, there was no evidence of microparticles contamination increasing along an urban gradient. About 20% of the microparticles found in mussels were plastics, specifically PET, PP, PS, and nylon. Quantifying spatial microparticles contamination is pertinent to both current and future water conditions in an economically and ecologically important region.

02.13.37 Nearness to Bottom Sediment, Vertically Stratified Water Metal Concentrations, and Metal Accumulation in Dragonfly Nymphs--A Field Caging Experiment

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Phylogenetically based patterns in metal accumulation have been noted in aquatic macroinvertebrates, but even closely related taxa can differ. For example, despite all being predaceous, different species of dragonflies inhabiting the same wetland or stream reach can accumulate substantially different concentrations of metals. The cause/s of this differential accumulation of metals is poorly known, but potential influences include differences in habitat use, feeding ecology, or physiology/biology. Previous studies established that aquatic nymphs of the dragonfly genus *Erythemis* accumulated higher levels of metals than *Anax* in a contaminated constructed wetland. Here we experimentally evaluated the potential influence of living at different water depths in a winter field-caging study. Our study site was a contaminated retention basin of the H-02 constructed wetland on the Department of Energy's Savannah River Site, SC, USA. Continual industrial effluents and episodic stormwater runoff flow through the retention basin. We caged *Erythemis simplicicolis* and *Anax longipes* at four water levels ranging from on the bottom to 75 cm above the sediment, near the water's surface. Water diffusion samplers were used to evaluate metal concentrations in water at each depth during the experiment. Metal concentrations in sediment were also evaluated. Both *Erythemis* and *Anax* accumulated higher Cu and Zn concentrations in the contaminated basin than nymphs from the source reference wetlands. Taxonomic differences in metal accumulation were maintained regardless of water depth. *Erythemis* accumulated higher levels of Cu and Zn than *Anax* at all water levels in the contaminated system, but the difference between taxa was reduced in the reference source wetlands. Vertical stratification of Cu and Zn in the retention basin water column was established with both metals generally at lower concentrations in deeper water. Whole body Cu and Zn concentrations of *Erythemis* were greatest at the top of the water column and lowest at the bottom. In contrast, Cu and Zn concentrations in *Anax* held at different water levels did not differ. Metal accumulation in *Erythemis* responded more to environmental differences and appeared to follow water concentrations in this experiment. This study illustrates the need for comprehensive studies when evaluating patterns of metal accumulation in aquatic biota.

02.13.38 Overview of U.S. EPA's Environmental Risk Assessment Approach for UVCBs in the New Chemicals Program Under TSCA: A Regulatory Perspective

J.S. Gallagher, U.S. Environmental Protection Agency / Office of Pollution Prevention and Toxics

The United States Environmental Protection Agency (U.S. EPA)'s New Chemicals Program manages risks to human health and the environment from new chemicals prior to their introduction into U.S. commerce under Section 5 of the Toxic Substances Control Act (TSCA). Of the greater than 400 new chemicals that were submitted to the U.S. EPA in 2020, approximately 6% are labeled as "unknown or variable composition, complex reaction products, and biological materials (UVCBs)." UVCBs are chemical substances "that have no definite molecular formula representation and either partial structural diagrams or no structural diagrams" and are challenging to test using traditional environmental toxicity test methodologies. This presentation will provide an overview on how the U.S. EPA assesses the environmental hazard and risk of UVCBs in the New Chemicals Program. It will discuss what approaches are taken for UVCBs when estimating environmental concentrations of concern (COCs) and risks in a data-poor environment. There will be a discussion about integrated approaches to testing and assessment (IATA) that combine existing information with the generation of new information to better understand the environmental risks of UVCBs. The latter part of the presentation will discuss current regulatory challenges when assessing UVCBs and propose a UVCB environmental testing strategy for hazard assessment purposes.

02.13.39 Oxidative Stress, Cyclooxygenase Inhibition and Behavioural Alterations: How Paracetamol Exposure Prompts Alterations on the Polychaete Hediste diversicolor

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Pharmaceuticals drugs are significant environmental stressors due to their worldwide use, inherent characteristics, and being usually released into the aquatic system without adequate treatment. Consequently, pharmaceutical drugs may affect several non-target aquatic species. Paracetamol is one of the most widely prescribed analgesics in human medical care. As a result, this compound is systematically reported to occur in the wild, where it may exert toxic effects on non-target species, which are mostly uncharacterized so far. The objective of the present work was to assess the acute (control, 5, 25, 125, 625 and 3125 µg/L) and chronic (control, 5, 10, 20, 40 and 80 µg/L) effects of paracetamol on behavioral endpoints, as well as on selected oxidative stress biomarkers [superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GRed)] and the anti-inflammatory activity biomarker cyclooxygenase (COX), in the estuarine Polychaeta species *Hediste diversicolor*. Exposure to paracetamol caused effects on behavioral traits, with increased burrowing time (96 h), and hypoactivity (28 days). In addition, exposure to paracetamol resulted also in significant increases of SOD activity, but only for intermediate levels of exposure, in both acutely and chronically exposed animals. Both forms of GPx had their activities significantly increased, especially after chronic exposure. Acutely exposed organisms had their GRed significantly decreased, while chronically exposed worms had their GRed activity augmented, but only for the lowest tested concentrations. Effects were also observed in terms of COX activity, showing that paracetamol absorption occurred and caused an inhibition of COX activity following both exposure regimes. It is possible to conclude that the exposure to concentrations of paracetamol close to the ones already found in the environment may be deleterious to marine ecosystems, endangering marine life by changing their overall redox balance, and the biochemical control of inflammatory intermediaries. Behavior was also modified, and the burrowing capacity was adversely affected. This set of effects clearly demonstrate that paracetamol exposure, under realistic conditions, is not exempt of adverse effects on marine invertebrates, such as Polychaeta species.

02.13.40 Physiological Impact of Arsenic and Uranium Mixture Exposure in Zebrafish

O. Lujan, C.R. Propper, M. Salanga, Northern Arizona University / Biological Sciences

Arsenic (As) is a naturally occurring element in the earth's crust with levels varying between locations, which can contaminate ground water and impact drinking water resources. The Navajo Nation for example contains unregulated wells that are used daily and may exceed current EPA maximum contaminant levels (MCL) of 10 µg/L As. Research has elucidated some of the effects of ingesting As contaminated water using translational models such as zebrafish and rats including developmental defects, nephrotoxicity, carcinogenicity, and an impairment in progeny development; however, the doses used in those studies are often many times higher than what is commonly encountered. Furthermore, toxicity from environmentally relevant levels of As in developmental systems is much less studied, and is the focus of our research. The fish embryo toxicity test (FET), developed by the Organization for Economic Cooperation and Development was utilized to test the hypothesis that low-dose arsenic exposure in embryonic zebrafish induces negative developmental outcomes. Briefly, embryonic zebrafish were raised in 24-well plates with one embryo per well containing 0 to 10 µM of sodium arsenite ($NaAsO_2$) and uranyl nitrate hexahydrate ($UO_2(NO_3)_2{\cdot}6H_2O$) individually or 0 to 1 µM of both elements combined. The developing zebrafish were scored for specific morphological points every 24-hours for a 96-hour duration. Although there was no evidence of acute toxicity, exposed zebrafish were unable to escape their chorion (i.e. hatch). Future work will assess the enzymatic activity of the hatching enzyme, cathepsin L, in zebrafish exposed to the compounds.

02.13.41 Prioritizing Organic Contaminants and Locations of Ecological Concern Using Sediment From Lower Rio Grande Valley Resacas

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Little is known about emerging 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. Nearly 75% of the region's wildlife habitat has been replaced by human development and agriculture which has led to increased concern about emerging contaminants and the impacts they have on wildlife and human health along the Arroyo Colorado Watershed. The LRGV contains about 810,000 ha of combined crop and citrus area with combined revenue of nearly 1.5 million US dollars from corn, cotton, and maize. The goal of this study is to identify and characterize new contaminant loading along the US-Mexico border and their effects on wildlife using them to survive. In this study, we collected sediment and water samples at 11 different sites from the LRGV resacas, performed chemical analysis of over 100 non-point source pollutants, and performed invertebrate, *Hyallela* and *Daphnia*, and vertebrate, fathead minnow and zebrafish, toxicity testing. Chemical analysis of water and sediment indicated there were resaca specific differences based on surrounding land-use patterns. Pyrethroids, such as L-cyhalothrin, permethrin, and bifenthrin were found at most sites, with concentrations ranging from 0.17 ppb to 19.99 ppb of permethrin at site 9. The most prevalent class of chemicals were fungicides followed by pyrethroids, demonstrating that legacy compounds such as organochlorines were less prevalent than previously thought. Toxicity data for invertebrates and both fish species will be presented as part of a toxicity identification evaluation for the prioritization of organic contaminants of concern. Zebrafish larvae behavior was analyzed by evaluating startle response from water collected at all 11 sites and found a statistically significant decrease in behavior from zebrafish larvae exposed to sites 1,2,3,4,8, and 11 compared to control.

02.13.42 Quantitative Analysis of Glyphosate, Glufosinate and AMPA Using In-Vial Addition of Pairing Agent

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Glyphosate and glufosinate are non-selective herbicides extensively used worldwide in agricultural and domestics applications. Therefore, these chemicals as well as AMPA (metabolite of glyphosate) are frequently detected in environmental samples. For decades, several approaches have been developed for the analysis of these compounds; however, their high polarity presents challenges for the development of robust analytical methods that require minimal sample preparation and eliminates derivatization steps. The work presented here focuses on the the analysis of glyphosate, glufosinate and AMPA using an innovative method based on reverse phase liquid chromatography/tandem mass spectrometry. The quantification of the pesticides' quantification is possible due to the in-vial addition of a pairing agent. With this approach, the quantity of pairing agent may be as low as 125 nmol per injection, what minimizes the classical disadvantages of ion-addition. In this presentation, the performance's results from this 7-minutes run time novel method in terms of separation, sensitivity and recoveries from selected sample types, will be shared.

02.13.44 Results of a Multigenerational Exposure of the Zebrafish (Danio rerio) to Perfluorooctane Sulfonate (PFOS)

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Repeatability of experimental results is fundamental to establishing confidence in observed outcomes. The present study sought to confirm the findings of an earlier investigation by Keiter et al (2012), which reported effects in a multi-generational exposure of PFOS on zebrafish growth at a concentration of 0.6 μg/L, among the lowest aquatic effect levels reported to date. Due to the implications for the use of the Keiter et al. data in deriving regulatory effect levels it is important to provide confirmation of these results. We are reproducing the study utilizing an expanded experimental design to include a larger, more narrowly spaced concentration range (0, 0.1, 0.6, 3.2, 20, 100 μg/L), higher replication ($n = 5$) with 50 fish per replicate, expanded analytical chemistry monitoring, and external validation of exposure concentrations to provide robust tests of experimental outcomes. Briefly, fish are being exposed through three generations in a flow-through apparatus, the parental (P1) and first familial (F1) generations exposed through 180 days post-fertilization (dpf) and the second familial (F2) generation exposed through 14 dpf. The P1 exposure is complete, and the F1 exposure is currently underway progressing through >100 dpf as of this abstract submission. Analytical chemistry for the P1 exposure indicated stability of PFOS concentrations in exposure water through the assay duration matching nominal concentrations within 12.5% on average. Independent analyses by a second analytical laboratory matched within 25% for all concentrations except 0.1 μg/L, which matched within 50%. No significant adverse effects ($p = 0.05$) on survivorship where observed at 10, 15, 20, 30, 60, 90, 111 and 180 dpf in the P1 exposure. Growth measured at 30, 60, and 90 days indicated a statistically significant decrease in body weight (compared to controls) in the P1 for the 100 μg/L exposure at day 60 (mean reduction of 21%), similar to the findings of Keiter et al. (2012), however, the significant decrease at 0.6 μg/L reported in that study was not observed. Finally, examination of eight weekly breeding trials with P1 fish (conducted starting on day 131) did not identify statistically significant negative effects of PFOS exposure on egg production or viability. The study is scheduled for completion in August 2021, and complete results will be presented. The contents of this abstract neither constitute nor necessarily reflect DOD or USEPA policy.

02.13.45 Spatial and Temporal Trends in Per and Polyfluoroalkyl Substances in Top Predatory Fish From Canada

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Per and Polyfluoroalkyl substances (PFAS) are chemicals of concern due to their persistence, and large number of compounds. PFASs are of particular concern in fish where concentrations of some compounds have lead to restrictions on consumption for both humans and wildlife. The current study outlines a scan of PFAS substances, including perfluoroalkyl carboxylates (PFACs), perfluoroalkyl sulfonates (PFSAs), fluorotelomer sulfonates and carboxylates, perfluorooctane sulfonamides, sulfonamidoacetic acids and ethanols, per and polyfluoroether carboxylates, and ether sulfonates in top predator fish. This study assesses spatial patterns of PFAS concentrations in top predatory fish from watersheds across Canada, with an emphasis on the Laurentian Great Lakes. Lake trout (Salvelinus namaycush) or walleye (Sander vietreus) were collected from sites across Canada from approximately 2006 to 2019 and analyzed for whole body concentrations of PFAS. A combination of annual sampling and retrospective analysis of archived samples was used to examine temporal trends in concentrations of PFAS in lake trout collected from two locations in the lower Laurentian Great Lakes (Lake Ontario) and Lake Huron. Concentrations of PFAS in predatory fish varied across Canada, with the greatest concentrations being observed in those collected from lakes located in areas of high population density; the lower Great Lakes-St. Lawrence River corridor. The highest concentrations of PFACs were found in the North Channel of Lake Huron, while the highest concentrations of PFSAs were in Lake Erie. PFACs were dominated by C8 – C11 compounds while PFOS was the most common PFSA. Concentrations of PFOS in the Lake Huron and Ontario increased from the early 1990s to early 2000s with subsequent declines, although elevated concentrations again being seen in some areas. In contrast, increases in concentrations of PFACs were seen more recently, within the past decade, in Lake Huron.

02.13.46 Sub-Lethal Metabolomics Comparison of Daphnia magna Responses to Legacy and Novel Perfluorinated Alkyl Substances (PFAS)

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Perfluorinated alkyl substances (PFASs) are a class of contaminants of concern due to their ubiquitous presence, persistence and bioaccumulative properties in organisms and in the environment. With over 5000 PFAS compounds produced globally, only a small number are routinely monitored in industrial effluents and in the environment. While legacy PFAS compounds such as perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) are still detected in environmental samples, alternative PFASs are often more widely detected. However, there is little information about how sub-lethal levels of PFASs may alter the health of aquatic organisms such as *Daphnia magna*. This uncertainty is coupled with the use of newer PFAS compounds, such as ammonium perfluoro(2-methyl-3-oxahexanoate) (Gen X), which are emerging as replacements for legacy PFASs. As such, this study examines how *D. magna*, a model organism in aquatic environments, responds to sub-lethal exposure to both legacy and new PFAS compounds and a PFAS mixture. Using liquid chromatography with tandem mass spectrometry, a targeted polar metabolic profile was quantified to determine perturbations in the exposure groups versus the unexposed organisms. Multivariate statistical analysis, partial least squares discriminant analysis (PLS-DA), demonstrated significant separation between the highest concentration treatments of PFOS, PFOA and PFAS mixture and the control group, suggesting a concentration-based dependence in the metabolic response of these contaminants. Further univariate statistical analyses compared the percent change of metabolites relative to the control. Several amino acid (serine, tryptophan, and histidine) and nucleoside (adenosine and uridine) concentrations were significantly altered due to the presence of the contaminants. Biochemical pathway analysis uncovered significant disruptions in the aminoacyl-tRNA biosynthesis pathway, and glycine, serine, and threonine metabolism pathways among all single contaminant exposures in *D. magna*. The mode of action of PFAS exposure in *D. magna* was also largely influenced on differences in the chemical structure between the different PFASs (alkyl chain vs. branching and sulfonic acid vs. carboxylic acid). The mode of action and metabolic perturbations of the single contaminants was not indicative of the response of the PFAS mixture. These results demonstrate that PFAS compounds and PFAS mixtures invoke different toxic responses in aquatic organisms.

02.13.47 Temporal Variation of Per- and Polyfluoroalkyl Substances (PFAS) in Fish, Surface Water, Sediment, and Prey Items from Historical AFFF Use Sites

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Per- and Polyfluoroalkyl substances (PFAS) are a group of fluorinated compounds widely used as ingredients in fire-suppressing Aqueous Film Forming Foams (AFFF). Several PFAS in particular, including perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and perfluorohexane sulfonate (PFHxS) are of paramount concern as persistent, bioaccumulative, and toxic (PBT) compounds as they accumulate rapidly in aquatic ecosystems. There is a lack of understanding regarding the temporal, spatial, and seasonal distribution of PFAS in aquatic ecosystems, presenting major implications for the uptake, accumulation, and ultimate fate of these compounds in biota, surface water, and sediment. This study aims to better characterize the bioaccumulation of PFAS in common freshwater fish species through the analysis of fish tissue, surface water, sediment, and prey items. These samples were collected from two sites with historic AFFF use, including the Recreational Pond at Willow Grove, Pennsylvania and Piscataway Creek at Joint Base Andrews, Maryland. We obtained biota and environmental samples at multiple locations within each site several times per week in Fall, Winter, and Summer, followed by an analysis of PFAS concentrations in fish liver, muscle, and gonad tissue as well as surface water and sediment samples. Our preliminary findings for samples collected from the Recreational Pond at Willow Grove suggest that concentrations of several PFAS including PFHxS and PFOA are variable in surface water and sediment on a multi-day scale. We also noted spatial variability with regard to magnitude and identity of PFAS within the Recreational Pond. Further, we observed relatively high concentrations of PFOS (>7,000 ng/g) and several other PFAS in fish tissue despite relatively low concentrations in water and sediment, suggesting high bioaccumulation of these compounds. Ongoing field work and chemical analysis will yield additional insights regarding bioaccumulation and temporal/spatial variability of PFAS in environmental media. This research is critically important to better our understanding of the temporal and seasonal variability of PFAS in environmental media to inform ecological risk assessments and better predict PFAS bioaccumulation in aquatic ecosystems.

02.13.48 The Effects of Estrogen and Ketoconazole on Expression of Bivalve Sex Determining Genes

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Mytilus edulis and other bivalves are used globally as sentinel organisms for monitoring pollution, such as endocrine disrupting compounds (EDCs). A characteristic effect of endocrine disruption is reproductive impairment, leading to skewed sex ratios, development of opposite sex characteristics, and population decline. Relatively low concentrations of EDCs can cause increased production of vitellogenin and intersex. Sexual development and natural sex ratios of *M. edulis*, the blue mussel, are being perturbed due to EDCs. In *M. edulis*, EDCs have been shown to cause feminized sex ratios in wild and lab mussels. In the New York Harbor, which is contaminated with EDCs, female sex determining genes were up-regulated, while male sex determining genes were down-regulated. Furthermore, the sex ratio was significantly skewed towards females. Though aromatase, the enzyme responsible for producing estrogen, has not been found in bivalves, they can respond to estrogen and similar compounds. One such synthetic estrogen, 17α-ethinylestradiol, has been shown to activate a gene related to oogenesis (VERL). This caused male mussels to overexpress this female-biased gene, indicating a feminization of male mussels. One of the current methods for identifying the sex of *M. edulis* uses a real time quantitate PCR assay that measures the difference in expression of two sex-specific genes: VERL (female) and VCL (male). However, this method requires that the mussel be sacrificed, and the mantle tissue be dissected. We expanded this assay to be non-destructive and use hemolymph, rather than mantle tissue. This assay can identify the sex of 71% of mussels, and the sex as determined by the assay is 100% accurate. We exposed male mussels to 50 ng/l 17α-ethinylestradiol (EE2), and female mussels to 30 µg/l ketoconazole (KZ), a steroidogenesis inhibitor, for 26 days. After the exposures, we determined the sex of each mussel again using mantle tissue to investigate effects of the EDCs on the sex of each mussel. Next, we measured expression of four sex determining genes: *DMRT1L, SoxH, FoxL2,* and β-*catenin*. EE2 caused a down regulation of male genes in males, and KZ dysregulated the entire sex determination pathway in females. This study aims to confirm that male mussels can change sex after an exposure to EE2 and to investigate how sex determining genes are affected by pollutants.

02.13.49 The Physiological Reactions of Daphnia magna When Dosed With Bifenthrin and Terbufos Insecticides

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Bifenthrin and Terbufos are both commonly used insecticides on crops throughout the United States. Bifenthrin functions by delaying the closing of voltage-gated sodium channels. Terbufos operates through loss of enzyme function allowing for an increase in acetylcholine neurotransmitter which can cause muscle contractions/paralysis. We hypothesized that Bifenthrin dosing will result in a physiological effect of decreased heart rate in *Daphnia magna*. We tested these hypotheses in laboratory controlled toxicity tests with Daphnia in glass jars with 100 mL of moderately hard water in a temperature controlled incubator set at 22 C. Our experiments were acute experiments lasting only 48 hrs. Our treatments were 1mL acetone dose (control), 0.1 ppb Bifenthrin, 1 ppb Bifenthrin, and 10 ppb Bifenthrin dosed using acetone as a carrier. Our preliminary results showed the average *D. magna* heart rate was 393 beats/minute in control, 393 beats/minute in 0.1 ppb Bifenthrin, 306 beats/minute in 1ppb Bifenthrin, and 377 beats/minute in 10ppb Bifenthrin. Only 1 ppb appeared to significantly affect the heart rate although data are still preliminary. We also hypothesized that non-lethal Terbufos dosing in *D. magna* will result in increased heart rate but have only completed data collection on Bifenthrin thus far. Our research seems to support that physiological effects of Bifenthrin and Terbufos insecticides can be measured through heart rate. Sublethal effects could be important if scaled up to the level of organismal and population level effects, so these low-level effects on *D. magna* could be important for organisms living in agricultural environment.

02.13.50 The Relationship Between PFAS and Stress in Bottlenose Dolphins from Indian River Lagoon, Florida and Charleston, South Carolina

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The ecotoxicological risks of per- and polyfluoroalkyl substances (PFAS) are incompletely understood. We investigated the associations between blood levels of free cortisol (an indicator of stress) and PFAS levels in bottlenose dolphins (*Tursiops truncatus*). We used parametric quantile regression models assuming a Weibull distribution of free cortisol conditional on PFAS, adjusting for the year and dolphins' age, sex, and location of sample [PFOS (n=192), PFOA (n=211), PFOSA (n=184), PFDA (n=211), PFDoDA (n=211), PFDS (n=88), PFHxS (n=211), PFHpA (n=85), PFNA (n=211), PFTA (n=102), PFTriA (n=77), and PFUA (n=211); for Bound PFOS (n=200), PFOA (n=219), PFOSA (n=191), PFDA (n=219), PFDoDA (n=219), PFDS (n=92), PFHxS (n=219), PFHpA (n=89), PFNA (n=219), PFTA (n=104), PFTriA (n=77), and PFUA (n=219)]. PFTriA was positively associated with free cortisol: dolphins had free cortisol levels 1.50 times higher (95% confidence interval: 1.08,2.09; Wald test p value = 0.015) in the middle tertile and 1.90 times higher (1.31,2.76; p=0.001) in the highest tertile relative to the lowest tertile of PFTriA exposure. Conversely, PFOS, PFOA, and PFHxS were all negatively associated with free cortisol: dolphins had free cortisol levels 0.74 times lower (0.57,0.97; p=0.028) in the middle tertile relative to the lowest tertile of PFOS exposure; 0.68 times lower (0.55,0.85; p=0.001) lower in the middle tertile relative to the lowest tertile of PFOA exposure; and 0.78 times lower (0.61,0.99; p= 0.047) in the highest tertile relative to the lowest tertile of PFHxS exposure. The following PFAS had no statistically significant associations with free cortisol levels: PFDS, PFOSA, PFHpA, PFNA, PFUnDA, PFDA, PFDoDA, and PFTA. The presence of both positive and negative associations among levels of different PFAS and blood cortisol levels suggests a more complicated relationship exists. Further research is needed to understand the effects of PFAS on dolphin stress physiology.

02.13.51 The Transcriptomic Basis of 3-Trifluoromethyl-4-Nitrophenol (TFM) Sensitivity in Fishes

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In the early 20^{th} century, sea lamprey (*Petromyzon marinus*) successfully invaded the Laurentian Great Lakes of North America resulting in severe ecological and economic damage. Lampricide applications in rivers, specifically 3-trifluoromethyl-4-nitrophenol (TFM), have been the principal sea lamprey control method. The main mechanism of toxicity of TFM in fishes is through an uncoupling of oxidative phosphorylation in the mitochondria. While TFM is relatively specific against sea lamprey, its toxicity can vary amongst fishes, which likely stems from the variability in the types of biotransformation genes in the genome, notably UDP-glucuronosyltransferases (UGT). However, the mechanism behind the differences in TFM sensitivity, particularly at the gene level, is poorly characterized. Thus, we sought to identify the potential molecular mechanisms underlying TFM toxicity and detoxification using a whole transcriptome approach (RNA-Seq) to examine differential expression of biotransformation gene targets in sea lamprey (i.e., target species) and bluegill sunfish (native species) exposed to TFM (species-specific 24h LC_{10}: lamprey = 2.21 mg L^{-1}, bluegill = 22.06 mg L^{-1}) over a 24 h period. We found that bluegill had a larger breadth of UGT gene families present in their transcriptome, relative to sea lamprey, and differential expression of UGTs was found only in TFM exposed bluegill. Differential expression of phase I, II, and III biotransformation genes were more diverse in bluegill when compared to sea lamprey. We also identified a broader mechanism of TFM toxicity at the transcript level, which included an arrest of cell growth, higher apoptosis, and immune responses in both species. Together, these data suggest that differences in TFM sensitivity in fishes may be linked to the species' transcriptomic response to exposure.

02.13.52 Toxicity of Candidate Fluorine-Free AFFF Replacement Formulations to Five Freshwater Species

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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 Department of Defense (DoD) is actively engaged in research to identify suitable replacements. The Strategic Environmental Research and Development Program (SERDP) is currently supporting a number of 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 the National Oceanic and Atmospheric Administration (NOAA) and the National Institute of Standards and Technology (NIST) and is focused on establishing acute and chronic toxicity of six candidate replacement formulations (e.g., 4 commercially available and 2 under development) in relation to a traditional PFAS containing formulation (Buckeye Platinum Plus). Acute toxicity of the candidate formulations were evaluated in five freshwater species, a benthic insect (Chironomus dilutus), an epibenthic crustacean (Hyalella. azteca), a pelagic invertebrate (Ceriodaphnia dubia) and two pelagic fish species (Pimephales promelas and Oncorhynchus mykiss). Chronic toxicity will be investigated using the most sensitive fish and invertebrate species. 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.

02.13.53 Toxicity of Wildland Fire-Fighting Chemicals in Pulsed Exposures

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During wildfire suppression, fire-fighting chemicals are sometimes directly applied to surface water. These applications result in relatively brief pulsed exposure to an initially high concentration that is progressively reduced as the retardant is diluted and washed downstream. A spill calculator was developed using the advection-dispersion model for use in estimating effects of applications of fire retardant. The model predicts distance and timing of downstream concentrations to allow for the assessment of the potential effects on threatened and endangered species and critical habitat. Maximum concentrations and durations of exposure were estimated from the model using real-world application data. To understand the potential toxicity of these events, 30-60 day post hatch rainbow trout (*Oncorhynchus mykiss*) were exposed to high concentrations of 1 of 4 different fire retardants, Phos-Chek® MVP-Fx, Phos-Chek® 259-Fx, Phos-Chek® LC-95A-R, or Phos-Chek® LC-95A-Fx. The concentrations tested varied for each chemical but included the highest concentration estimated by the model from applications during fire-fighting activities. Mortality was monitored every hour for the first 8 hours of exposure and again at 24 hours. Survival of the trout varied by chemical tested and exposure duration with some mortality occurring by 30 minutes of exposure at the highest concentrations tested. These data can be used by resource managers in selecting products for application to manage any potential risks to aquatic organisms associated with application to water.

02.13.54 Trace Elements in Soft Tissues and Shells of Invasive Bivalves in Lake Michigan

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Bivalves have long been used as biomonitor organisms for trace element pollution in aquatic systems. A typical biomonitor study either compares sites relative to a potential source of pollution, or monitors changes in concentrations at one or more sites over time. The underlying assumption is that trace element concentrations in tissue vary primarily due to exposure, however, factors such as organism growth (allometry), reproductive status, and environmental components such as sediment composition have all been known to affect bioaccumulation of trace elements in soft tissues. This makes comparisons between sites difficult, since many of these factors change from site to site. The current study investigated trace element accumulation in invasive quagga and zebra mussels in Lake Michigan. Evidence for trace element correlation with allometric and sediment particle size variables was found in quagga mussels sampled in a spatially intensive manner, although tissue concentrations did not correlate to concentrations of the same trace elements in the surrounding sediments. This suggested that factors other than exposure via sediment were primarily responsible for variation in tissue trace element concentrations. To explore this further, more temporally intensive sampling was performed on zebra mussels. This sampling covered the zebra mussel reproductive period and sampled populations not directly exposed to sediment, in order to eliminate potential effects of sediment particle size and chemistry. Zebra mussels demonstrated weaker correlations to allometric variables in both tissues and shells. The shells were shown to have lower variability in trace element concentration over both time and space. This suggests that zebra mussel shells may act as a more consistent source of biomonitor data for trace elements.

02.13.55 Twelve Road Deicers Have Varying Effects on Zebrafish Survival and Development

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One threat fresh water faces is salinization from road deicers. The use of the most common deicer, sodium chloride (NaCl), has greatly increased and so too have the ecological concerns. Because of this, new products are being developed to mitigate the effects; however, the environmental effects of many are unknown. Therefore, we investigated the effects of 12 deicers from three groups: single-chloride [NaCl, $MgCl_2$ (magnesium), $CaCl_2$ (calcium)], chloride-based mixtures (organic additives, multiple chloride salts, catalyst, acetates), and salt-free urea-based. Lab-grade (≥99%) chloride salts were also included for comparison. To investigate the developmental and neurodevelopmental effects of the deicers, we used zebrafish (*Danio rerio*) embryos, an emerging tool to study contaminants. We studied survival and development across six concentrations of each deicer (1.0, 2.5, 5.0, 7.5, 10.0 ppt, plus a control) and three exposure windows [embryonic (0-24 hours post fertilization, hpf), larval (102-126 hpf), and prolonged (0-126 hpf)]. The embryonic and larval exposures had few effects: in the embryonic exposure, only the lab-grade $MgCl_2$ and the acetate mixture (15-40% NaCl) had large effects; while in the larval exposure, only lab-grade $MgCl_2$, $MgCl_2$ + organic additive, and $MgCl_2 + K_2SO_4$ had large effects. In the prolonged exposure, the urea-based deicers had no effect; however, at least one concentration of all other deicers affected zebrafish survival and development, including increased abnormalities and decreased hatching. Lab-grade $CaCl_2$ and $CaCl_2$ + organic additive affected the zebrafish at all five concentrations tested. We also compared deicers at similar conductivities and found that the lab-grade and single-chloride deicer NaCl had a smaller effect (e.g., abnormalities) than $CaCl_2$ (e.g., severe abnormalities to failed hatching), which had a smaller effect than $MgCl_2$ (e.g., severe abnormalities to mortality). Additionally, deicers NaCl + $MgCl_2$ + KCl + catalyst and the acetate mixture had almost no effect, while the $MgCl_2 + K_2SO_4$ had huge effects on survival. In conclusion, all deicers except the urea-based deicers affected zebrafish mortality and development. Some containing NaCl were less detrimental despite single-chloride NaCl deicers being one of the few without environmentally friendly claims. Therefore, alternatives to NaCl need to be investigated further to fully understand their impacts. *This abstract does not necessarily reflect EPA policy.*

02.13.56 Understanding the Effect of Mitochondrial MicroRNAs in Response to Wastewater Effluent Stressors in Freshwater Fish (Etheostoma spp.)

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The effect of metabolic stress on fish is well-studied, though specific epigenetic mechanisms guiding that response are not fully understood. MicroRNA are known to regulate transcript abundance in fish and have recently been shown to translocate into the mitochondria of mammalian cells where they influence expression of mitochondrially encoded genes; however, this has not been studied in fish. Mitochondria are essential to aerobic respiration, and mitochondrial DNA encodes subunits for proteins involved in oxidative phosphorylation (OXPHOS). This research seeks to understand how mitochondrially localized microRNA (mitomiRs) influence mitochondrial gene expression and function in freshwater fish under metabolic stress. The Grand River in Waterloo, Ontario is an outlet for wastewater treatment plant (WWTP) effluent containing a variety of pollutants, which is a known metabolic stressor to darters (*Etheostoma spp.*). To understand if their physiological response to WWTP effluent is regulated by mitomiRs, darter species living upstream and downstream of the Waterloo WWTP were collected. Bioinformatic analyses identified candidate mitomiRs that are predicted to target mitochondrial mRNAs, then transcriptional and enzymatic analyses were completed in mitochondria-rich livers. It was hypothesized that 1) mitomiRs and their target mRNAs will be differentially regulated in fish exposed to metabolic stress compared to control conditions, resulting in altered OXPHOS enzyme activity and whole organism metabolism, and 2) mitomiR profiles will differ among species with different tolerances to metabolic stress. Indeed, downstream of the WWTP, mitomiRs let-7a and miR-1 were upregulated in mitochondrial fractions of rainbow darter livers, and cytochrome c oxidase activity was significantly decreased. Further investigation could identify the specific mechanisms of mitomiRs in fish exposed to metabolic stressors, and how they specifically target and regulate mitochondrial gene expression.

02.13.57 Uptake and Effects of Two Understudied Per- and Polyfluoroalkyl Substances (PFAS) on Early Life Stages of the Estuarine Fish, Red Drum (Sciaenops ocellatus)

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Per- and polyfluoroalkyl substances (PFAS) are a large class of manmade organofluorine compounds that are used in a variety of industrial and commercial applications. These compounds are highly mobile, persistent, and ubiquitously detected in sediment, soil, surface waters, wildlife, and humans. Although contamination can be attributed to a wide variety of origins, PFAS-containing aqueous firefighting foams (AFFFs) are recognized as the primary source of PFAS in the environment. Accordingly, many types of PFAS are routinely detected in media surrounding military installations, airports, fire training centers, and industrial sites where petroleum fires may pose a risk. Estuarine habitats along the Gulf of Mexico are particularly vulnerable to PFAS contamination from a variety of sources (including AFFF), yet toxicological studies examining the effects of PFAS on aquatic biota overwhelmingly utilize freshwater models. Moreover, many commonly detected PFAS remain chronically understudied, with little to no ecotoxicological data available for evaluating ecological risk in impacted estuaries. Therefore, the purpose of this study was to examine the uptake and effects of two understudied PFAS, perfluorohexanesulfonic acid (PFHxS) and perfluorooctane sulfonamide (PFOSA), on early life stage (ELS) estuarine fishes. PFHxS and PFOSA were selected as PFAS of interest because of their high frequency of detection, bioaccumulative potential, relative contribution to total concentrations in impacted media, and the paucity of ecotoxicological data available for these compounds. ELS red drum (*Sciaenops ocellatus*) were selected as an estuarine model for the present study, due to their abundance in Gulf of Mexico, as well as their commercial and ecological importance. ELS drum were exposed to a range of PFHxS or PFOSA concentrations for 28 days, with uptake and developmental endpoints assessed at regular intervals. Results of the present study address important gaps in our knowledge regarding the potential ecological risks associated with PFAS contamination in estuarine systems.

02.13.58 Using Biological Responses to Monitor Freshwater Post-Spill Conditions Over 3 Years in Blacktail Creek, North Dakota, USA

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In January 2015, a pipeline carrying unconventional oil and gas (UOG) wastewater spilled approximately 70,000 barrels (11 million liters) of wastewater into Blacktail Creek, North Dakota, USA. This presentation describes a multi-level investigative approach used to assess the potential effects of oil and brine spills on aquatic life. In this study we used a combination of fish 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. Microbial community and estrogenicity analyses were conducted on collected sediments. Combining fish toxicological, microbial community structure and estrogenicity information provides a complete ecological investigation that defines potential influences of contaminants at organismal, population, and community levels as compared to isolated studies using single approaches. In situ bioassays have implications for the individual survival and changes at the population level, microbial community structure defines potential changes at the community level and estrogenicity measurements define changes at the individual and molecular level. 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 compared to reference sites 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, NH_4T 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. Therefore, early biological effects were defined with changes at the individual, population and community levels of biological organization. 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 activity of ammonia from shallow ground water.

02.13.61 Zebrafish (Danio rerio) Pronephric Function Affected by Deepwater Horizon Crude Oil Exposure: Renal Fluid Clearance Capacity and Glomerular Filtration Integrity

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Teleost fish exposed during early life stages (ELS) to *Deepwater Horizon* (DWH) crude oil manifest developmental defects. Those associated with developmental cardiotoxicity are believed to elicit a characteristic suite of downstream effects (e.g., pericardial and yolk sac edema) and likely impair the proper development of other organs. As evidence, zebrafish (*Danio rerio*) larvae acutely exposed to DWH crude oil showed transcriptional changes in key genes involved in early kidney (i.e., pronephros) development and function. These molecular alterations are further coupled with pronephric morphological defects, which could lead to physiological impairments. Notably, the pronephros is an important osmoregulatory organ, and the onset of its osmoregulatory function, fluid filtration and clearance capacity appear to be relatively early in teleost fishes. Any defects associated with kidney development and function are likely associated with a decreased ability of the organism to osmoregulate and potentially adapt to challenging osmotic environments, leading to mortality if the insult is severe. The objective of this study is to examine the acute effects of crude oil exposure during zebrafish ELS on the pronephros function by assessing its fluid clearance capacity and filtration integrity. Following exposure to DWH high energy water-accommodated fractions (HEWAF) of oil, zebrafish will be injected into the cardiac venous sinus with either fluorescein-labeled (FITC) inulin to assess pronephric clearance capacity, or with FITC- 70-kDa dextran to assess glomerular filtration integrity. Fluorescence will be quantified after the injection at 1, 6, 24 and 48 hours post injection by capturing images under a fluorescence microscope and analyzing fluorescence intensity with ImageJ. We expect that data obtained from these experiments will demonstrate a reduced pronephric fluid clearance capacity and altered glomerular filtration size selectivity in zebrafish previously exposed to DWH crude oil. This research was made possible by a grant from The Gulf of Mexico Research Initiative.

02.20 Late Breaking Science: Aquatic Toxicology, Ecology and Stress Response

02.20.01 Assessing Comparative Toxicity of Legacy Phthalates and Their Replacements Using Embryo-Larval Zebrafish

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Phthalic acid esters (PAEs) are plasticizers that have become ubiquitous and are considered as contaminants of emerging concern. These chemicals are known to induce endocrine disruption, developmental deformities, cardiac toxicity, oxidative stress, eventually affecting survival. European Union's Registration, Evaluation, Authorization and Restrictions of Chemicals (REACH) has restricted the use of legacy phthalates. The United States Environmental Protection Agency (USEPA) has listed six legacy phthalates on the Priority Pollutant List. Owing to their hydrophobic nature, they pose a greater concern through bioaccumulation and trophic transfer; hence, newer phthalate replacements were introduced in the market—which are now widely used. To understand the effects of these replacements, it is important to conduct a comparative toxicity assessment for legacy phthalates and their replacements. In this study, we aimed to assess acute toxicity of legacy phthalates: dibutyl phthalate (DBP), butyl benzyl phthalate (BBP), di(2-ethylhexyl) phthalate (DEHP), dioctyl phthalate (DnOP), di-isononyl phthalate (DINP), dimethyl phthalate (DMP); and their replacements: 1,2-cyclohexane dicarboxylic acid diisononyl ester (DINCH), acetyl tributyl citrate (ATBC), and di-(2-ethylhexyl) adipate (DEHA). Zebrafish were exposed to phthalate concentrations ranging from 0.01 nM to 10 µM, at or below water soluble concentrations, with DMSO 0.1% (v/v) as solvent. We assessed the morphology and white light startle response of zebrafish larvae exposed to phthalates and their alternatives from 0–5 days post-fertilization. Our results showed that BBP and DBP were most toxic with respect to survival and BBP had highest incidences of total morphological deformities. There was no concentration-dependent statistically significant difference to white light startle response for both legacy and replacement phthalates. The findings of this study will contribute to our understanding of the potency of phthalate replacements and the safety of the products containing these replacements.

02.20.03 Biotransformation Capacity of Alternative Test Systems: Detoxification of Electrophiles via the Mercapturic Acid Pathway in Zebrafish Embryos and PAC2 Cell Line

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New approach methodologies (NAMs) allow reducing animal use in toxicity testing for environmental risk assessment. In 2013, OECD adopted the zebrafish (*Danio rerio*) embryo acute toxicity test (TG No. 236) as an alternative to tests with adult fish. This year, the RTgill-W1 cell line assay for acute toxicity (TG No. 249) became the first OECD-adopted NAM based on a permanent fish cell line derived from gills of rainbow trout, *Oncorhynchus mykiss*. In turn, zebrafish cell lines could offer a useful alternative to testing in warm-water fish. Correct interpretation of data obtained with alternative models requires understanding of their biotransformation capacity, as biotransformation differences could affect test outcomes for certain chemicals. Available evidence does suggest these models' ability to biotransform some chemicals, but their biotransformation capacity has not yet been characterized systematically. To start addressing this gap, we here studied the mercapturic acid pathway, an important phase II biotransformation route involved in the clearance of electrophilic compounds and phase I biotransformation products, in zebrafish early life stages and the PAC2 cell line derived from 24h-old embryos. This pathway starts with conjugation of an electrophile with glutathione, catalyzed by glutathione S-transferases (GSTs). The initial glutathione conjugate is then biotransformed to the respective mercapturate, which is highly hydrophilic and hence easily excreted. This transformation occurs through a series of spatially separated steps catalyzed by integral membrane enzymes, γ-glutamyl transferases and peptidases, followed by intracellular N-acetyl transferases. Using mass spectrometry-based targeted proteomics, we demonstrated that both models express a broad repertoire of GST enzymes, with distinct patterns shown by different GST classes. We further showed that both test systems are able to biotransform a model electrophile, 1-chloro-2,4-dinitrobenzene (CDNB), for which the majority of intermediates as well as the final mercapturate could be detected. These findings confirm the functionality of the mercapturic acid pathway in both systems, thus supporting their suitability for testing electrophile chemicals. Our ongoing work aims to apply the developed approach to address other enzyme families involved in biotransformation, such as CYPs or sulfotransferases, and will cover rainbow trout cell lines in addition to zebrafish-based models.

02.20.04 Combined Effect of Polystyrene Microplastics and Diazepam on Fish Social Behavior

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Microplastics have been widely detected in the aquatic environment. In addition, environmental pollution by pharmaceuticals, which disrupt fish social behavior, is frequently occurred. Therefore, the combined effects of microplastics and pharmaceuticals on fish social behavior is required to be investigated. This study evaluated the combined effect of polystyrene microplastics (PS-MS, 2 µm diameter) and diazepam, a benzodiazepine antidepressant, on medaka (*Oryzias latipes*) social behavior. Five test group were set up for this experiment: solvent control group, PS-MP exposure group (0.04 mg/L), low concentration diazepam exposure group (0.03 mg/L), high concentration diazepam exposure group (0.3 mg/L), and co-exposure group of PS-MP and low concentration diazepam. Medaka (four-month post hatching, weight 348.7 ± 52.9 mg) was exposed to PS-MS and/or diazepam for 7 days (exposure period), then replaced into the polystyrene microplastics and diazepam-free water and maintained for 5 days (recovery period). Shoaling behavior test, which observes medaka leaving behavior from the shoal, was conducted on each last day of the exposure and the recovery period. During the exposure period, the leaving behavior from the shoal in the PS-MP exposure group, the high concentration diazepam exposure group and the co-exposure group was significantly higher than that in the solvent control group. Even during the recovery period, leaving behavior from the shoal in the high concentration diazepam exposure group and the co-exposure group was observed frequently comparing to the solvent control group. This result suggested that the combined effect of PS-MS and diazepam induced the leaving behavior from the shoal in the co-exposure group, and this combined effect lasted to the end of the recovery period. In addition, PS-MS was accumulated in medaka of the co-exposure group and remained in the gastrointestinal tract of medaka in the recovery period. These results suggest that the desorption of diazepam from microplastics remaining in the gastrointestinal tract may affect medaka social behavior. This study showed that combined effects of PS-MP and diazepam frequently induced the medaka leaving behavior from the shoal: the suppression of medaka social behavior. Our result suggested that the co-existence of microplastics and pharmaceuticals might strongly induce the adverse effect of chemical pollutants on fish social behavior in the aquatic environment.

02.20.05 Comparative Acute Toxicity of PFOA and its Replacement GenX in Embryo-Larval Zebrafish

K. Satbhai, J. Crago, Texas Tech University / Department of Environmental Toxicology

Perfluoroalkyl substances (PFAS) are receiving a great attention due to their persistence in the environment and the adverse effects they pose on ecological and human health. After phasing out C8 PFAS, newer, shorter length replacement compounds have been introduced—such as perfluoro (2-methyl-3-oxahexanoic) acid (GenX). There is not enough data on the toxic effects of replacement compounds; hence, it is important to understand the persistence of these chemicals as well as the exposure levels and their effects on organisms. We conducted a comparative analysis of Gen X and perfluorooctanoic acid (PFOA) using embryo-larval zebrafish (ZF). For the 24 hours post-fertilization (hpf) survival assay, the LC_{50} for GenX was 169.99 µM while the LC_{50} for PFOA was 81.84 µM. No significant effects were seen in morphological deformities (tail kink, yolk sac edema, pericardial edema) at concentrations below the respective LC_{50} values for each chemical. At 54 hpf, GenX but not PFOA showed an increase in hatching success. At 5 days post-fertilization (dpf), no statistically significant differences were seen in white light startle response. Furthermore, we assessed the uptake from 0–5 dpf, and depuration from 5–7 dpf. Our overall findings demonstrate that GenX is less acutely toxic than PFOA to embryo-larval zebrafish.

02.20.06 Consumption of Microplastics by Small Saltwater Fishes and Potential Human Exposure: Case Study in Coastal Areas of Binh Dinh Province, Vietnam

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An intensive investigation of microplastics in the digestive tract of small fishes in the coastal areas of Binh Dinh province, Vietnam was carried out for the first time. The local fish species chosen for the present study include Goby (*Glossogobius aureus* and *Oxyurichthys ophthalmonema*), Anchovy (*Stolephorus commersonnii*), Pompano (*Decapterus macrosoma*), Goatfish (*Upeneus moluccensis*), and Hering (*Sardinella gibbosa*). The fishes were collected from four areas along the coast of Binh Dinh province: the Thi Nai lagoon in Quy Nhon city and Tuy Phuoc district, the De Gi beach in Phu Cat district, the Xuan Thanh beach in Phu My district, and the Tam Quan Bac beach in Hoai Nhon town for microplastic analysis. The samples were collected twice, in December 2020 (the end of the rainy season) and in March 2021 (the beginning of the dry season) with respect to all the chosen areas. Depending on their appearance in different coastal areas and seasons, three of the same or different fish species with 10 individuals each were collected for investigation. Microplastics were characterized by concentration, size, shape, color, and intrinsic nature. The results showed that all fishes consumed significant amounts of microplastics. The concentration ranged from 5.7 ± 2.7 to 23.9 ± 9.4 particles/fish. Among the microplastic types, fibers with the average length of 800 – 900µm were the dominant and accounted for 74.5%, followed by fragments (25.5%) with the average area of 90,000 – 135,000μm^2. Yellow microplastics were dominant (35.72%), followed by blue (13.73%), black (5.42%), green (4.49%), purple (2.78%), pink (2.28%), brown (1.94%), orange (0.47%), and red (0.13%). Results of the present study reveal that microplastic contamination in small saltwater fishes along the coastal areas of Binh Dinh is relatively high in comparison with other regions examined worldwide. The presence of microplastics in the digestive system of these fishes poses a potential microplastic transfer to high trophic fish through the food chain and microplastic exposure to people in Vietnam, especially for coastal communities as they often consume whole body of small fishes.

02.20.07 Design of a Protocol to Evaluate Effects of Microplastic Particles in Cladocerans

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Microplastic (MP) contamination is considered a growing problem in terms of its production and observed impacts on various organisms. In this sense, numerous studies have provided information on the behavior and effects of MPs in aquatic biota, however, there are shortcomings in terms of the methodology applied and differences related to the use of surfactants and the modality (static or dynamic) used in each study. This work focuses on the design of a protocol to evaluate the effects of polyethylene (PE) MP on Dapnia magna and to provide evidence on the acute (mortality-48h) and chronic (life cycle- 21 days) impact of different sizes of MPs. First, the use of surfactant tween 20 (0.1 and 0.05% v/v) and the static and dynamic study modalities (Twist Shaker TW3 (FINEPCR), VWR Shaker model 3500 and Silogex MS-M-S10) were evaluated. Neonates, juveniles and adults were exposed to increasing concentrations of MPs including actual concentrations reported for the aquatic environment. Results indicated that the use of Tween 20 (0.1% v/v) is required for particles between 45-53 and 212-250 µm, being optional for smaller sizes. The static mode was the most appropriate for all particles. Significant differences was observed in mortalitly (%) between neonates, juveniles and adults ($p< 0.05$). Smaller particles (1-5 and 27-32 µm) significantly affected the life cycle of D. magna ($p < 0.05$), reducing the number of offspring compared to the control. Finally, the effect of the MP in cladocerans would depend on the concentration of particles, the exposure time, the size of the organisms and above all the size of the particle studied. This work is an important contribution to future research evaluating the adverse effects produced by MP, demonstrating that these emerging pollutants are effectively ingested by cladocerans, and might be able to transfer these particles to higher-level organisms and produce a negative impact on the trophic chain.

02.20.08 Developing a Comparative Understanding of the Aquatic Toxicology of (±) Anatoxin-a and (+) Anatoxin-a in Two Common Fish Models

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Harmful algal bloom toxins present risks to public health and the environment. Anatoxin-a is a globally found neurotoxic cyanobacterial toxin that affects growth, reproduction, and survival of aquatic organisms. Few studies have examined influences of anatoxin-a on gene expression and behavior with common fish models. Therefore, after performing probabilistic hazard assessments, here we studied larval fathead minnow and zebrafish responses following exposure to (±) anatoxin-a at environmentally relevant, analytically verified concentrations We found duration and distance of fathead minnows swimming at the highest speed (>20 mm/s) was significantly lowered (ANOVA, $\alpha = 0.1$) by (±) anatoxin-a in 0.1, 1, and 1.5 mg/L treatments, 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, and DNA damage. While there was little change in zebrafish, fathead minnows showed upregulation in many neurotoxicity related genes, though only *elavl3* was significant (ANOVA, $\alpha = 0.05$) as well as downregulation of *gst* and *cyp3a126*. This suggests fathead minnows may be more sensitive to this toxin based on these endpoints. We subsequently have repeated these studies with (+) anatoxin-a, previously reported as the only enantiomer produced by cyanobacteria and observed differential sensitivities among endpoints and between organisms with high mortality in fathead minnows >0.5 mg/L concentrations and showing a largely refractory response in zebrafish. Comparative understanding of cyanotoxins is necessary to support robust assessments and management of algal blooms.

02.20.09 Does Harmful Algal Bloom Exposure Exacerbate the Impacts of Cold Stress in Florida Manatees?

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The Florida manatee (*Trichechus manatus latirostris* is a population with individuals reported to migrate west to summer foraging sites along the northern Gulf of Mexico (nGoM) coast and return to warm water refugia in Florida in winter. Manatees have a very low tolerance to water < 20° C and often exhibit symptoms of cold stress syndrome (CSS) after prolonged exposure to such temperatures. Based on prior studies we know that manatees bioaccumulate toxins produced by harmful algal blooms (HABs) throughout the nGoM, likely from dermal and oral exposure pathways. Etiologies for manatees stranded along the nGoM coast in the winter are often declared to be due to CSS, but the role of HAB toxin exposure in CSS individuals has not been evaluated. We propose that the underlying mechanisms for CSS-related deaths of manatees in AL and MS may be exacerbated by HAB toxin burdens. Little is known about the combined effects of these toxins with other stressors in manatees, so in this study we evaluate exposure pathways, HAB toxin diversity distribution in CSS animals (via ELISA and LC-MS), and perform a re-evaluation of necropsy data. These combined data will estimate the toxin loads in manatee tissues and their food sources to determine routes and timing of exposure and the effects of toxins combined with other health conditions (e.g., CSS). The findings of this study may support the reassessment of cause of death from prior standings, allowing for the revision of risk assessments for this nGoM population. Gaining a better understanding of the health risks posed by multiple toxin classes will result in a more informed species conservation effort and plan.

02.20.10 Effect of Cadmium and Salinity Stressor Synergy on Vallisneria neotropicalis

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In recent decades, many species of macrophytes are in decline across the Gulf coast due to a variety of stressors including changing salinity regimes and heavy metal pollutants. Changing salinity places ionic and osmotic stress on submerged macrophytes, and in particular affects freshwater to oligohaline regions of estuaries where the plants have little tolerance for salt. Heavy metals such as cadmium (Cd) can have a variety of detrimental effects on submerged macrophytes such as oxidative stress. Less understood is how combined multiple stressors interactions, such as combined increases in salinity and heavy metal concentrations affect submerged macrophytes and their corresponding microbial community. In this study we evaluate the combined effect of salinity and Cd on Vallisneria neotropicalis, a submerged macrophyte that inhabits the oligohaline range of the estuary. Salinity and Cd stressors will be modulated in a three by three factorial experiment over the course of twelve weeks. Vallisneria shoots will be planted in individual mesocosms. Each treatment will be replicated a total of three times. The Cd effect in Vallisneria will be measured via growth rate and fluorometry. V. neotropicalis has a salinity tolerance up to 5 ppt after which the measured growth rate is expected to be impaired. In treatments with high salinity conditions, the combined effect of Cd and salinity is expected to be more than additive, as the higher concentration of chloride ions is expected to make otherwise sulfide bound Cd more bioavailable. In summation, the work will advance our understanding of multiple stressors impacts on V. neotropicalis and inform resource managers on the consequences of present environmental stressor trends of increasing salinity and heavy metals.

02.20.11 Effects of Carbamates on Vertebrate Behavior and Neurodevelopment

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Carbaryl is a common pesticide used in the U.S. for combating insect infestations. When carbaryl is used, it often contaminates natural habitats. Having contaminated habitats frequently results in vertebrate animals being exposed to these chemicals. Previous studies have shown that carbaryl, a carbamate pesticide, reversibly inhibits acetylcholinesterase, affecting movement and muscle function, and at high doses can lead to uncontrollable urination, seizures, or even death. However, it is unclear how low dose exposures, more commonly encountered in nature, impact the health and physiology of vertebrates. Using amphibians as a model to test the effects of low dose carbaryl exposures, we exposed Pickerel Frog (Lithobates [Rana] palustris) tadpoles to concentrations of 0, 1 and 10 ppb of carbaryl in a double blind controlled laboratory experiment. After two weeks of exposure, three behavioral assays were performed to test boldness, olfactory sensitivity, and muscle movement. The specimens were then euthanized and weighed. Both body and brain measurements were taken and analyzed. Results showed that there was an effect of 1ppb carbaryl on the size of the diencephalon. There were no effects of 10 ppb carbaryl on the brain. Data analysis on behavior is ongoing. There is still more to learn about the effects of low dose carbaryl exposures, however, this study provides the first evidence that carbaryl has low dose and non-monotonic effects on the vertebrate brain.

02.20.12 Evaluating In Vitro Mechanisms of Endocrine Disruption by Marine Algal Toxins

S.C. Collins, University of South Alabama/ Dauphin Island Sea Lab

Marine algal toxins and endocrine disrupting chemicals (EDCs) have been reported to cause similar behavioral, morphological, and reproductive effects in marine biota, but it is not yet clear the mechanisms that are related. The responses of anthropogenic EDCs such as pesticides have been well defined and suggested to be based on androgen and estrogen receptor regulation. However, the mechanisms that cause these effects have not been directly studied for marine algal toxins. With the prevalence of marine algae increasing on temporal and spatial scales, potential exposure may be significant as chronic and sublethal effects are largely unexplored. In this study, we evaluated the effects of a variety of algal toxins on androgen transcriptional activity using a cell-based reporter assay for the detection of (anti-)androgenic activity via expression of a luciferase reporter gene, allowing dose dependent detection via bioluminescence. Likewise, estrogen receptor effects were evaluated *in vitro* using a cell line transfected with an estrogen response element, also tied to a luciferase response. Ciguatoxins, maitotoxins, okadaic acid, and microcystins were evaluated in AR and ER agonist and antagonist assays. Interestingly, our results showed no effect on AR transcriptional activity from the toxins when compared to positive androgen agonist (dihydrotestosterone) and antagonist (hydroxyflutamide). This contrasts with previous studies that show marine toxins causing endocrine disrupting effects, which leads us to believe that there is a different mechanism involved in AR disruption. Evaluation of ER disruption by marine biotoxins is now underway to rule out ER mediated responses. Based on these data, we have initiated a controlled *in vivo* exposure in *Fundulus grandis* to evaluate other mechanisms and effects of marine toxins on the endocrine system.

02.20.13 Experimental Determination of the Isotopic Fractionation of Cadmium in Eastern Oyster Shells

J.J. Pavelites, University of South Alabama/ Dauphin Island Sea Lab / Department of Marine Sciences

Cadmium (Cd) is a toxic heavy metal that bioaccumulates in aquatic ecosystems. It can be difficult to determine if Cd comes from natural or man-made sources. This study aims to develop oyster shell as a site-specific indicator of Cd sources. Oysters are excellent potential bioindicators of Cd exposure because they remain in one location after setting and incorporate Cd in their shells. A determination of source could then be made using the oyster shell's isotope ratio because anthropogenically sourced Cd is lighter than natural sources. To determine if oysters can reliably be used to define Cd sources, we must quantify any fractionation (alteration of the ratio of isotopes) that may occur during uptake into oyster shells. Juvenile Crassostrea virginica will be cultivated in an enclosed circulating system and grown in the presence of a known ratio of Cd isotopes. A control group of oysters in untreated water will be reared in tandem with the treated system. Oysters will be sampled from the treated and control systems every two weeks for three months. The oysters will then be dissected with the shell and internal organs stored separately. The shell's margin of new growth added during the experimental period will be removed and dissolved in inorganic acid before being passed through ion-exchange resins to remove impurities. The resulting sample will be analyzed by Inductively Coupled Plasma – Mass Spectrometry to determine the ratio of 114Cd/110Cd . The magnitude of biological fractionation will be quantified by comparing the isotope ratios in shell to the ratio in treatment water from experimental tanks. The ability to determine the source of Cd contamination has applications in environmental accountability and mariculture. Industrial contamination could be more easily tracked and implicated in assessments of seafood safety along with organismal and ecosystem health. Future studies could build on this research to compare fractionation in shell between oysters exposed to contaminated source water and oysters fed contaminated food particles.

02.20.14 High Throughput Assay Development with Raphidocelis subcapitata

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To keep pace with the introduction of new chemicals to the marketplace, it is important to develop rapid, cost-effective, and efficient assays to evaluate the hazard(s) those chemicals may pose to ecosystems and human health. While an abundance of high throughput assays for evaluating potential hazards to human health have been developed, less attention has been focused on the evaluation of ecological hazards, particularly to organisms like invertebrates and plants whose physiology is less conserved with that of humans. Current studies use varying algal species, in different containment styles, and are generally longer than 24 hours. This presentation describes the development of a 96-well plate-based high throughput assay with the green algae, *Raphidocelis subcapitata*. Exposures were conducted with two chemicals per plate on 3 replicate, deep well 96-well plates (1 mL per well). To keep algae in suspension, exposures were conducted on a shaker under constant growth lighting. Apical observations (cell count, photopigments, and cytotoxicity) were taken using a microplate reader after pelleting the algae, dewatering, and resuspending in fresh growth media. Using BMDExpress2 software the data was used to calculate benchmark doses (BMDs). The resulting BMDs were generally higher than those reported by existing publications. This methodology is still being evolved to reduce technical error, increase efficiency, and increase the accuracy of high throughput exposures. *The contents of this abstract neither constitute, nor necessarily reflect, official USEPA policy.*

02.20.15 Impact of Fluorine-Free Replacement Formulations on Clinical Chemistry and Hematology Parameters

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Previously conducted reproductive/developmental tests with per-and polyfluoroalkyl substances (PFAS) have shown effects on thyroid hormones, immune function, and neonatal survival. In response to the National Defense Authorization Act’s (NDAA) prescribed phase-out of PFAS-containing, aqueous film- forming foams (AFFFs), the use of PFAS-free mixtures are being explored. These novel formulations are expected to be less environmentally toxic and persistent than PFAS, yet follow a similar surfactant polymer design. As part of a toxicity evaluation, we conducted acute and subacute toxicity tests in CD1 mice (*Mus musculus*) using six fluorine-free formulations, along with one C6-containing formulation as a positive control. Clinical chemistry and hematology parameters impacted by PFAS were analyzed for direct comparison against the PFAS-free formulations. Clinical chemistry parameters included albumin, total protein, alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea nitrogen, creatinine, cholesterol, glucose, and triglycerides. Hematology parameters included total red blood cell (RBC) count, total white blood cell (WBC) count with differential, hemoglobin, hematocrit, platelets, and reticulocytes. Analysis of these parameters will prove crucial in determining whether these novel AFFFs are less toxic than PFAS.

02.20.16 In Vitro Evaluation of Cytochrome P450 1 Inducibility by Exposure to TCDD and FICZ in Finless Porpoise Fibroblasts

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Finless porpoises (*Neophocaena asiaeorientalis*) inhabit the Japanese coasts and form five subpopulations. Their habitats have been threatened by the contamination with dioxin-like compounds (DLCs) for decades. DLCs activate the aryl hydrocarbon receptor (AHR) and induce transcription of multiple AHR target genes including xenobiotic metabolizing enzymes such as cytochrome P450 1 isoforms (CYP1) in animals. AHR activation induces endocrine disruption, developmental failure, and immune dysfunction. CYP1 induction is thus a marker of AHR-mediated toxic effects. However, due to ethical and legal constraints, the toxicological study on cetaceans is still challenging. The aim of this study is to evaluate the induction potencies of the CYP1 gene by AHR ligands using fibroblasts derived from finless porpoises. Fibroblasts were cultured from six finless porpoises in the Seto Inland Sea, Omura Bay and Ariake Sound subpopulations in Japan that were found dead during 2016 - 2019. This sample set consists of both sexes with different growth stages. Fibroblasts were exposed to graded concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and an endogenous AHR ligand, 6-formylindoro[3,2-b] carbazole (FICZ). The expression levels of CYP1A1, CYP1A2, and CYP1B1 in fibroblasts were measured by real-time qPCR. The concentrations of dioxin-like coplanar polychlorinated biphenyls in their blubber were also analyzed, and were converted to TCDD Toxic Equivalents (TEQs). TCDD dose-dependently induced CYP1A1 and CYP1B1 expressions in finless porpoise fibroblasts, whereas no expression of CYP1A2 was detected. For all individuals, induction potency of CYP1A1 by TCDD exposure was higher than that of CYP1B1, demonstrating that CYP1A1 is a useful biomarker for DLCs exposure in the fibroblasts of this species. The individual differences in the half maximal effect concentrations (EC_{50}) of CYP1A1 induction by TCDD exposure were less than 10-fold, suggesting less effect of the subpopulation, sex, and growth stage of porpoises on CYP1A1 induction. Since the EC_{50} values were closed to the TEQs calculated from the blubber concentrations, toxicity of DLCs via AHR and CYP1 of finless porpoises is of concern. The CYP1A1 inducibility by FICZ exposure varied with the time of exposure and was notably sensitive to the short-term exposure, suggesting that FICZ may be metabolized by induced CYP1A1, but may play an important role of the physiological function in the fibroblasts.

02.20.17 Influence of Sampling Methods and Seasons on Selenium Enrichment and Trophic Transfer in Boreal Lakes

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Selenium is an essential micronutrient known to bioaccumulate and occasionally biomagnify in aquatic food webs. Few studies have investigated the influence of sampling methods and seasonal differences in Se bioaccumulation at the base of the food chain. Additionally, the effects of low water temperature associated with prolonged ice-cover periods on Se uptake and further transfer to benthic macroinvertebrates (BMI) have been overlooked. The aim of this study was to verify potential differences related to sampling methods (artificial substrates *vs* sediment grab samples) and seasons (summer *vs* winter) in Se enrichment and transfer to BMI sampled from boreal lakes downstream from a Saskatchewan milling operation. During summer 2019, water, sediment grab samples (*n=3*) and artificial substrates (*n=4*) were sampled from 10 sites with different degrees of effluent exposure. In winter 2021, water and sediment grab samples (*n=3*) were sampled at 4 of those 10 sites. Water and sediment samples, and periphyton growing on top of the sediment and scraped from artificial substrates, were analyzed for total Se concentrations. Benthic macroinvertebrates were sorted to the lowest taxonomic level and subsequently analyzed for total Se concentrations. Enrichment functions (EF) in periphyton and trophic transfer factors (TTF) in BMI were calculate for both sampling methods and seasons. Overall, the artificial substrates exhibited a greater BMI diversity relative to sediment grab samples. Periphyton scraped from the artificial substrates presented higher Se concentrations (3.2 ± 2.6 µg/g d.w) relative to those collected with sediment grab samples (1.0 ± 1.2 µg/g d.w). At higher effluent exposure sites, mean Se concentrations in BMI were consistent among sampling methods, whereas significantly lower Se concentrations were observed in BMI sampled with sediment grab samples at medium and low exposure sites. Total Se concentrations in water varied among sites and, except for the highest effluent exposure site (near the effluent diffuser), were higher in winter (0.06 ± 0.04 µg/L) relative to summer (0.03 ± 0.06 µg/L). Selenium concentrations in sediment were consistent among seasons, but significantly higher Se concentrations in periphyton were observed in winter. Nevertheless, mean Se concentrations in BMI sampled in winter (2.9 ± 1.9 µg/g d.w) were comparable to those sampled in summer (1.8 ± 2.2 µg/g d.w). Future investigations on Se bioaccumulation should consider different sampling strategies and seasons to better estimate potential risks to aquatic biota.

02.20.18 iTrackDNA: Non-Destructive Precision Genomics for Environmental Impact Tracking in a Global Climate Change Era

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The analysis of environmental DNA (eDNA), i.e., genetic material shed from organisms into their environment, is highly promising as eDNA detection from environmental samples provides rapid, non-destructive, accurate and cost-effective biodiversity information. However, inconsistent practices and poor quality eDNA detection tools threaten End-User (regulators, industry, Indigenous Peoples, NGOs) uptake because of unacceptably high false negatives and false positives that can compromise effective management decision-making. iTrackDNA is a new multi-year, large scale applied research project that aims to address these concerns with researchers and End-Users across sectors in North America. It will build End-User capacity through innovative, accessible, socially responsible genomics-based analytical eDNA tools for effective decision-making by: 1) supporting the creation of a targeted eDNA detection national standard; 2) building eDNA kits to detect 100 priority invertebrates, fish, amphibians, birds, reptiles, and mammals in coastal and inland ecosystems; 3) applying 10 eRNA kits for determining animal biosurveillance, biosanitation, and bioremediation effectiveness; 4) generating decision support software for modeling regional biodiversity changes integrating Indigenous Ecological Knowledge; 5) developing an eDNA training, certification, and inter-lab validation framework for consultants, researchers, regulators, and managers; and 6) producing a guidance document on eDNA-based methods integration into management, policy & regulations. iTrackDNA has an unprecedented, broad stakeholder commitment to enable eDNA standards adoption, policy development, and transformative testing to confidently enable eDNA applications in coastal and inland ecological surveys and biosurveillance for mining, forestry, energy, and infrastructure projects; economically important, at-risk, and invasive species management; and climate change impact tracking. Supported by Genomes Canada, British Columbia, and Québec, and 26 industry, government, FN, and NGO partners

02.20.19 Maternal Exposure to Bisphenol S Induces Neuropeptide Signalling Dysfunction, Oxidative Stress, and Abnormal Social Behaviours in Zebrafish Offspring

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Bisphenol S (BPS) is one of the main substitutes of bisphenol A that is increasingly used in a wide range of industrial and consumer products. Recent studies show that BPS induces multiple adverse effects; however, the transgenerational impacts of BPS remain largely unknown. In the present study, adult female zebrafish (*Danio rerio*) were exposed to environmentally relevant concentrations of BPS (0, 1, 10, 30 µg/L) and 1 µg/L of 17-β-estradiol (E2) as a positive control for a period of 60 days. Female fish have then paired with control male zebrafish and their offspring were collected and raised in control water for 6 months. Our results indicated that maternal exposure to BPS influences male offspring's social behaviour and anxiety responses in a dose-specific manner. The group preference and social cohesion were significantly decreased by 1 and 10 µg/L, respectively. Meanwhile, the number of excursions increased at high levels of BPS (30 µg/L) and E2. Maternal exposure to 1 and 30 µg/L BPS and 1 µg/L E2 decreased the stress responses of offspring in the novel tank test. The impaired social behaviours were associated with dysregulations in the level of neuropeptide signalling system (AVT levels at 10 µg/L) as well as the transcription of genes involved in the neuropeptide signalling system (*AVT*, *avt1r1b*) and antioxidant enzymes (*cat* and *Mn-sod*). Collectively, these results suggest that maternal exposure to the environmentally relevant concentrations of BPS disrupt zebrafish offspring social behaviours, likely by inducing oxidative stress and disrupting the neuropeptide signalling system in offspring.

02.20.20 Microplastics in Freshwater State of Science Review and Pilot Sampling Program in Yukon Territory, Canada

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Microplastics (MP) in water and aquatic ecosystems are a growing concern for which there is little data, especially in northern freshwater environments. Preliminary screening conducted by Yukon Government's Water Resources Branch (WRB) and Bruce Porter has indicated the presence of MP in Yukon's waterways. In response, Core Geoscience Services (CoreGeo) and WRB conducted a state-of-the-art study to test sampling and laboratory methodologies towards identifying standard practices for analysis of MP. Based on the findings of the literature review, discussions with laboratories, and environmental conditions, a pilot study was designed to sample for MP in the Yukon River upstream of the Takhini River confluence. Samples were collected using two methods on March 24, 2021. Grab samples (n=12) were obtained by pouring 100L of river water through a set of two sieves (8" brass 45µm and 500µm). Filter samples (n=5) were obtained using a submersible pump and controller pumping 100L of river water through an in-line 0.45µm high-capacity groundwater filter. QA/QC samples (blanks and controls) were also collected. Sample analyses were conducted at four different laboratories and included microscopy particle count particle size distribution and elemental composition. Both sampling methods tested presented some challenges, particularly for winter sampling, and with contamination. Comparison and interpretation of laboratory results are also challenging with no standard analytical method for MP. Results show that MP are likely present in the Yukon River downstream of Whitehorse. Recommendations for future sampling include simple grab samples in more replicates and additional sampling locations to better understand the sources and fate of MP in the environment.

02.20.21 PFOA in U.S. Ambient Surface Waters: Occurrence in Aquatic Environments

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The U.S. Environmental Protection Agency (EPA) is actively deriving Perfluorooctanoic acid (PFOA) Ambient Water Quality Criteria (AWQC) for the protection of aquatic life. A thorough review and compilation of PFOA occurrence data in U.S., ambient surface waters is an important consideration in development of the Problem Formulation section of AWQC. The goal of this review was to obtain and summarize PFOA occurrence data from U.S. ambient surface waters. Most of the current, published PFOA occurrence studies have focused on a handful of broad geographic regions, many times targeting sites with known manufacturing or industrial uses of PFASs, such as the Great Lakes, the Cape Fear river and waterbodies near Decatur, Alabama. Concentrations of PFOA in U.S. surface waters vary widely, with observed concentrations ranging over seven orders of magnitude and detected generally in the pg/L to ng/L range, with some sites having reported concentrations in the µg/L range. PFOA concentrations in surface water appeared to increase with levels of urbanization. For example, across the Great Lakes region, PFOA was higher in the downstream and relatively urbanized lakes of Erie and Ontario and lower in the upstream lakes of Superior, Michigan, and Huron. Overall, these studies show the distribution and variability of PFOA concentrations in U.S. surface waters and provide greater context to publicly available PFOA toxicity data that may be used to derive criteria or inform environmental risk assessments.

02.20.22 Physiological Effects of Wastewater Exposure on Chinook Salmon

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Wastewater Treatment Plants are known to release toxicants and contaminants of emerging concern into the aquatic environment via wastewater effluent (WWE); however, effects of chemical mixtures in WWE are still poorly understood. Chinook salmon (*Oncorhynchus tshawytscha*) are important to Pacific Northwest culture and as a food resource for critically endangered southern resident killer whales (*Orcinus orca*). Chinook abundances have declined drastically across the Pacific Northwestern U.S. in recent decades. One source of stress is chemical pollution from WWE. This study investigated effects of WWE on juvenile Chinook health by measuring changes in brain function, osmoregulation, endocrine disruption, and stress following 10 days of exposure. We hypothesize that WWE exposure will decrease cortisol, increase vitellogenin, and decrease brain and gill sodium/potassium ATPase activity. Findings from this study will help us better understand which effects of wastewater exposure are most harmful and need to be addressed via improved wastewater treatment.

02.20.23 Recommendations for Improving the Reliability and Reproducibility of Ecotoxicoproteomics

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Technological advances in instrumentation over the past decade have fueled leaps in scientific innovation- opening the door to a world of high throughput and data intensive New Approach Methodologies (NAMs) that aim to incorporate *in vitro*, *in silico*, and omics techniques into ecotoxicology to meet rising demands for toxicity data. Nevertheless, regulatory agencies and risk assessors have been slow to incorporate these techniques into existing frameworks citing concerns for a lack of standardization, reproducibility, transparent data analysis, and interpretation. Proteomics is one such new approach tool which uses mass spectrometry to detect sensitive sublethal shifts in protein expression. This tool exhibits a high level of applicability to diverse questions and can identify targeted, modified, global, and differential protein expression patterns. As proteins are the direct mediator of phenotype and cellular response, proteomic techniques are attractive tools that can feed into the development of biomarkers of affect and Adverse Outcome Pathways (AOPs), a tool which aids in the design of testing strategies to support safety assessments allowing the strategic prioritization of testing. However, unique challenges arise when applying proteomic techniques to non-mammalian tissue types, non-model organisms, and toxicological workflows. These challenges are compounded with poor consensus across methodologies and reporting guidelines in the field, which has not kept pace with proteomics standards across other disciplines.In the current analysis, we critically examine recent journal articles in which proteomics is used as a tool to investigate toxicological response in model fish species. By characterizing the strengths and gaps of current ecotoxicoproteomics applications and providing strategic recommendations to improve the reliability and reproducibility of this tool, we aim to support establishment of best practices to facilitate further development of this emerging area.

02.20.24 Subchronic Impacts of 2,4-D Herbicide Weedestroy®AM40 on Associative Learning in Juvenile Yellow Perch (Perca flavescens)

B. Anton, University of Wisconsin, Madison / Forest and Wildlife Ecology

Aquatic herbicides are commonly used to control a wide variety of invasive and nuisance plants. One common active ingredient used in commercial herbicide formulations in Midwestern states is 2,4-dichlorophenoxyacetic acid (2,4-D). Due to the stability of 2,4-D in aquatic environments, many non-target aquatic species experience prolonged exposure throughout critical developmental life stages that can affect essential behaviors. However, the impacts of 2,4-D exposure on learning behaviors in juvenile fish are poorly understood. Therefore, we conducted a series of experiments using a maze environment to determine the effects of a commercial 2,4-D amine salt herbicide formulation (Weedestroy®AM40; WAM40; at 0.00, 0.50, 2.00, and 50.00 mg/L 2,4-D acid equivalent (a.e.)) exposure on juvenile yellow perch's ability to perform a feed associated learning behavior. We observed a significant decrease in the ability of yellow perch to correctly complete the feed associated learning behavior within 200 s when exposed to WAM40 at 2.00 and 50.00 mg/L 2,4-D as compared to controls ($p = 0.0002$; $p < 0.0001$, respectively) and within 600 s when exposed to WAM40 at 2.00 and 50.0 mg/L 2,4-D as compared to the controls ($p = 0.0107$ and $p < 0.0001$). These data suggest that exposure to 2,4-D in WAM40 can both increase the amount of time it takes for yellow perch to complete a feed associated learning behavior and/or obstruct the behavior altogether. Further experiments showed no significant decreases in locomotion ($p > 0.05$), hunger motivation ($p > 0.05$), and a visually guided startle response ($p > 0.05$), in all treatment groups tested as compared to controls. This suggests that 2,4-D in WAM40 does not inhibit feed associated learning behaviors via interaction with these mechanisms. Altogether, the results indicate that the use of 2,4-D herbicides for weed control in aquatic ecosystems could present risks to cognitive functions that control essential behaviors of yellow perch.

02.20.25 Temporal Trends of Halogenated Flame Retardants in Herring Gull Eggs in Canada Between 2008 and 2019

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Halogenated flame retardants are chemicals of international concern. As a result, some have been regulated under the Stockholm Convention on Persistent Organic Pollutants (SC-POPs). To address whether such international regulations are resulting in declines of halogenated flame retardants, we examined concentrations of polybromodiphenyl ethers (PBDEs), hexabromocyclododecane (HBCDD), and dechlorane plus (DP) in herring gull (*Larus argentatus*) eggs collected from pan-Canadian colony sites between 2008 and 2019. Eggs were sampled from 18 colonies located in the Laurentian Great Lakes (n = 10), St. Lawrence River (n = 3), Atlantic (n = 2), and Arctic (n = 2). One egg was sampled annually or biannually from 10-15 individual nests from each colony for 5-12 years. Eggs were pooled on an equal wet weight basis and analysed using gas chromatography-mass spectrometry for 14 PBDE congeners, total-a-HBCDD, and *syn*- and *anti*-DP. We used generalized linear models and breakpoint analysis to determine if, and when, changes in concentration occurred and the direction and magnitude of these changes. Our results show that in nearly all cases, a breakpoint occurred in the trend indicating a shift in chemical concentration over time and that largely, concentrations of these flame retardants are decreasing across Canada. Frequently, trends followed an increase-to-decrease pattern, indicating that emissions of these flame retardants have decreased as their production and use have been phased out. For HBCDD and BDE-209, changes in trends occurred between the date of nomination and the date of listing, indicating that nomination and subsequent risk assessments and reviews of DecaBDE and HBCDD is driving decreases in concentrations seen in Canada, rather than the date of listing. For the lower brominated PBDEs, trends varied greatly, indicating that in some colonies the regulations on Octa- and Penta-BDE commercial mixtures are resulting in decreases in concentrations, while in others emissions are still occurring due to stockpiles or discarded products, or re-release of environmental reservoirs. Decreases were also seen for DP even though this product has not been internationally regulated. Overall, these results suggest that management due to the SC-POPs has affected concentrations of flame retardants seen in Canadian herring gulls, though emissions are still occurring due to the persistent nature of these chemicals.

02.20.26 The Effects of Bisphenol-A on the Survival and Morphology of D.excentricus Larvae

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Bisphenol-A (BPA) is an ingredient used in phenol resins, epoxy resins, polyesters and polycarbonate plastics. BPA gets released into the aquatic environment through toxic waste disposal, groundwater, sewage runoff and plastic leaching. When BPA enters aquatic environments, it can affect the development and physiology of marine organisms. This study aimed to understand the effects of relatively low concentrations of BPA on the larvae of the sand dollar *Dendraster excentricus*. Embryos and larvae were exposed to four treatment solutions (no additives, vehicle control, 50 μg/L BPA, and 500 μg/L BPA) for 7 days. The two BPA treatment concentrations were higher than have been detected in coastal marine waters, but lower than concentrations used in previous studies of BPA effects on echinoderm development. After exposure, larval midline body length, postoral arm length, frequency of normal development and survivorship were measured. Midline body length, frequency of normal development and survivorship all decreased in the presence of BPA, in a dose-dependent manner. This study showed relatively low concentrations of BPA can impact the development of sand dollar larvae.

02.20.27 The Use of Fish Scale Hormone Concentrations in the Assessment of Long-Term Stress in Teleost Fish

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Integumentary structures such as fish scales incorporate steroid hormones as they grow. The hormone concentrations of the fish scale should thus be representative of the activity of hormone releasing processes such as the stress response over long periods of time. This is advantageous when attempting to quantify long-term stress and related adverse effects. Current data on fish scale hormone concentration is limited to cortisol, the major stress mediator in teleost fish. However, many compounds are involved in the stress response and it is their net effect that determines the ensuing health status of the organism. Our objective was therefore to extract two additional stress related hormones, dehydroepiandrosterone (DHEA) and cortisone, allowing us to generate multi-hormone values and glean further information on stress status. This was accomplished by stressing adult rainbow trout once daily using one of three randomly applied physical stressors for 14 days. Hormone concentrations in both scale and serum were then quantified and compared to control fish that were left undisturbed. We found that all three stress hormones were significantly elevated in stressed fish scales. Multi-hormone analyses such as the cortisol:DHEA ratio and total glucocorticoid concentration represented by cortisol+cortisone content were also significantly different in the stressed fish scales. Notably, there were no significant differences found in serum hormone concentrations between stressed and control fish, supporting the use of fish scales in place of other media when assessing long-term stress in teleost fish.

02.20.28 Tissue-Specific Alterations of the Lipidome in Oysters by Estrogenic Mixtures

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Estrogens are present as complex mixtures in wastewater effluents, from both human and industrially derived sources with evidence of estrogen-mediated reproductive effects in biota residing in receiving waters such as vitellogenin induction and gonadal dysgenesis. As a filter feeder, the oyster is a vulnerable species in marine and estuarine environments that can bioaccumulate estrogens. However, little is known on the effects of estrogens on sub-lethal effects on metabolic functioning. This study aimed to evaluate possible alterations of lipidomic metabolites in Sydney rock oysters, *Saccostrea glomerata*, exposed to "low" and "high" estrogenic mixtures of relevant concentrations observed in receiving waters in Australia and globally. A lower abundance of many non-polar metabolites was exhibited after 7d acute exposure followed by ^{1}HNMR analysis. Despite a low exposure of 0.0002% (v/v) ethanol as the carrier solvent, a strong solvent effect was exhibited, with reduced lipidic metabolites, particularly in the gill and digestive gland. The male gonad did not experience any significant alteration of the lipidome by the estrogenic mixture exposure; however, in the female gonad, significant reductions of phospholipids and phosphatidylcholine was observed after exposure to high estrogenic mixtures only. We hypothesise that the lower abundances of these phospholipids in the female gonad may be attributable to: 1) lower algal consumption and thus lower uptake of lipidic metabolites; 2) lower availability of substrates for the synthesis of phospholipid and phosphatidylcholine; and/or 3) the generation of reactive oxygen species via estrogen metabolism, and consequent lipid peroxidation.

03.01 Advances in Aquatic and Terrestrial Plant Ecotoxicology and Risk Assessment

03.01.02 Assessment of Boron Toxicity on Seedling Emergence, Growth and Vegetative Vigour of Monocotyledon and Dicotyledon Plants

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Increasing agricultural production demands use of chemicals like pesticides and insecticides. The rampant use of these chemicals due to lack of knowledge has played havoc with environment and human health. It has several deleterious effects on crop production and hence, toxicity of these chemicals should be assessed for appropriate use. In agriculture, boric acid (source of boron) is used as a pesticide, insecticide, herbicide, and fungicide. Boron is an essential plant micronutrient that plays crucial role in plant growth and development. Boron in soil and irrigation water plays an important role in agricultural production. It has an extremely narrow range between deficiency and toxicity. Boron toxicity has been recognized as a serious problem in arid and semi-arid regions of the world affecting the yield of agricultural plants. Boron requirement differs in plant species. The available soil boron deficient for one crop may be toxic to another. In this study, we evaluated the effects of sub-toxic application rates of boron on seedling emergence, growth, and vegetative vigour of two monocotyledon (corn and wheat) and two dicotyledon (mung bean and cucumber) plants. Seeds were sown in soil and boron levels ranging from 0.0 (control), 0.5, 1.0, 2.0, 4.0, and 8.0 kg boron/hectare were applied using boric acid on to the soil surface. Emergence and phytotoxic effects were observed up to 14 days post 50% emergence of the seedlings in the control group. Endpoints measured were visual assessment of seedling emergence, shoot length and dry shoot weight. To assess the effect of boron on vegetative vigour, all 4 species of plants were grown up to 2-4 true leaf stage and boric acid was sprayed on foliage at boron levels ranging from 0.0 (control), 0.6, 1.2, 2.4, 4.8, and 9.6 kg boron/hectare. Phytotoxicity was observed up to 21 days post exposure. Endpoints measured were shoot length and dry shoot weight. The results indicated concentration dependent decrease in emergence, shoot length and shoot biomass as compared to control plants of all species. Phytotoxic effects observed were chlorosis, leaf burn and stunted growth of seedlings in the higher boron concentrations as compared to normal growth in control plants. Thus, this study indicates boron toxicity at higher application rates to corn, wheat, mung bean and cucumber plants.

03.01.03 Chloride Toxicity to the Daphnid (Daphnia magna) and Duckweed (Lemna gibba) in Natural and Reconstituted High Hardness Prairie Pothole Waters

D. Harper, A. Farag, U.S. Geological Survey / Columbia Environmental Research Center - Jackson Field Research Station

Oil and gas production in the Bakken Shale region in North Dakota and Montana, USA has generated large quantities of produced wastewater. This water is the single largest waste stream associated with oil and gas production. Wastewater from oil and gas production in the Bakken formation is highly saline (>250,000 mg/L TDS) and with a high proportion of Na and Cl. The Bakken formation lies within the Prairie Pothole region, an area with thousands of perennial and seasonal wetlands, ponds, lakes, and streams which provide critical wildlife habitat for migratory birds and other wildlife. The water chemistry of natural surface and groundwater within the region has relatively high water hardness (>300 mg/L as $CaCO_3$). We investigated the effects of Cl on *Daphnia magna* and *Lemna gibba* in reconstituted hard water simulating the surface waters of the Prairie Pothole region and in waters collected from the region. Acute toxicity (48-h LC50) for Cl in hard water to *D. magna* ranged from 3,788 mg/L in reconstituted waters (hardness 550 as CaCO3) to 3,070 in field collected waters (hardness 3,152 as CaCO3). The 7-d Duckweed IC50 for Cl ranged from 2,531 mg/L in reconstituted waters (hardness 550 as CaCO3) to 2,441 (hardness 3,152 as CaCO3). Very high hardness in the field collected waters did not affect sensitivity to Cl in these exposures.

03.01.04 Impacts of Amendments to Promote Mine Revegetation: Relationships Among Effects on Tailing Chemistry and Douglas Fir Health and Needle Chemistry

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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 water and soil 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 mining tailings where it is difficult to establish vegetation. We evaluated the effects of amending the tailings with lime, biochar, biosolids, and locally-sourced microbes (LSM) on tailing chemistry, and plant survival and needle responses. This research evaluated plant response data across tailing amendments for both greenhouse and field studies with Douglas fir (*Pseudotsuga menziesii*). "Heat maps" based on Pearson correlation coefficients, were used to evaluate relationships among tailing chemistry, seedling survival and injury and concentration of metals in needles. The analysis indicated that in both the greenhouse and field studies, seedling survival was positively related with an increase in tailing pH, decreased electrical conductivity, and decreased tailing concentrations of metals (Al, Cu, Fe, Mn, Zn). Seedling injury was positively related to needle Al and Cu concentrations in the field study, but seedling injury and needle metal concentrations were less strongly correlated in the greenhouse study. Tailing and needle metal concentrations showed similar patterns of correlation in both the field and greenhouse studies. 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 enhance plant growth by improving soil chemistry, thus increasing the potential for success of vegetation reestablishment to degraded mine soils in Superfund sites across the United States.

03.01.05 Addressing Variability in EPA-FIFRA Non-Target Terrestrial Plant Testing

C. Habig, Compliance Services International

EPA typically requires non-target terrestrial plant (NTP) testing for registration of outdoor-use pesticide products. Two laboratory greenhouse tests are generally required, seedling emergence testing and vegetative vigor testing. Testing is required on multiple species for both tests. Spacing between test concentrations (rates) ranges from 2-fold to 4-fold. Primary test endpoints include percent emergence, percent survival, and plant growth (height and weight). Point estimate test endpoints used for risk assessment are expressed as an EC_{25} and a NOEC for each species; the EC_{25} is used to evaluate potential risks to non-endangered species, while the NOEC is used to evaluate potential risks to endangered species. EC_{25} values for each test species are calculated using regression analysis and the NOEC is calculated using hypothesis testing compared to the controls. However, the guideline design for both tests, and particularly the seedling emergence test, often results in high variability within a test group (including the controls) and between test groups within a test. There is also frequently high variability between tests conducted under different greenhouse conditions. This high variability results in considerable uncertainty in the EC_{25} and NOEC values, and therefore, the resulting risk assessments. This high variability and uncertainty is generally not acknowledged in lower-tier NTP risk assessments. Clear dose-response curves may not occur with data for a given species, resulting in large confidence bounds on the EC_{25}. Similarly, the variability may result in a poorly defined NOEC, due to either a lack of statistically significant differences despite 10-20% inhibition of a test endpoint or due to inverse dose responses, with greater effects occurring at lower test concentrations than higher test concentrations. Examples of several datasets will be presented, with discussion of different approaches and interpretations of these data sets, and how these interpretations impact the potential off-site endangered species and non-endangered species risk assessments for herbicide products.

03.01.06 On the Influence of Uncertainty on SSD Analyses - Case Studies With Non-Terrestrial Target Plants

S. Charles, University Lyon 1 / Laboratory of Biometry and Evolutionnary Biology; V. Ducrot, Bayer Ag / Environmental Safety - Environmental Effects

Today, SSD analyses are a key tool for Tier 2 environmental risk assessment of chemicals. They provide a reliable assessment of the range of sensitivities within plant or animal communities and allow to estimate the hazardous concentration prone to affect 5% of the species (HC5). An HC5 estimate can be obtained from the fit of a probability distribution on a sufficient collection of toxicity values from a statistically sound analysis. Toxicity values are derived from the fit of a regression model on toxicity test data observed at several treatment levels at a target time point. This fit provides toxicity values as point estimates, associated with an uncertainty interval, either a confidence or a credible interval. Nevertheless, this uncertainty is rarely accounted for in ERA. In the case of non-terrestrial target plants (NTTP), treatment levels are called tested rates. The toxicity test data analysis leads to the estimate of 50% Effective Rates (ER50), that are then used as inputs for the SSD analysis. Up to ten species are usually considered for ERA of herbicides. The used tested rates are selected prior to the experiments with little prior knowledge on the sensitivity of species to the herbicides. Consequently, unbound values (ER50 < lowest tested rate or ER50 > highest tested rate) can occur instead of uncertainty intervals, especially when the range of tested rates does not match the sensitivity of a species or when this species is not affected at the highest application rate. The Guidance Document on Terrestrial Ecotoxicology (GDTE) provides no advice on how to deal with neither uncertainty intervals nor unbound ER50 values within SSD analyses. As a consequence, the common practice is to ignore the uncertainty by considering point estimates, and either to discard unbound ER50 values or to substitute them with arbitrary values. Such practices constitute a loss of valuable information with drawbacks: (i) the range of remaining ER50values may not cover the full range of sensitivities; (ii) if unbound ER50 values occur for many species, after discarding them, the data set might not be sufficient enough to allow the SSD analysis to be performed. In this presentation, based on several case studies, we will show how it is possible to account for both the uncertainty based on bounded intervals for ER50 values and unbound ER50 values into SSD analyses. In particular, we will explore their influence on the HC5 estimate.

03.02 Ecotoxicity of Short-Chain Fluorinated and Fluorine-Free Firefighting Formulations

03.02.01 Screening Level Hazard Ranking in a Data-Poor Environment: A Case Study Using Fluorine-Free AFFFs

A. East, L. Holden, U.S. Army Public Health Center / Toxicology; A. Narizzano, M. Quinn, U.S. Army Public Health Center / Toxicology Directorate

Replacing PFAS-containing aqueous film forming foams (AFFFs) with products of high hazard is a concern for human and environmental health and could delay the transition from legacy AFFFs. However, evaluation of products based on insufficient hazard data remains a core issue for risk assessment. To address this issue, we evaluated environmental hazard data and several hazard ranking methods using a selection of data-poor fluorine-free AFFFs. We approached ranking using endpoints with experimental and predicted data while evaluating data availability and ecological relevance. The final ranking emerges from various multivariate ranking metrics and scoring systems. We found a combination of principal component analysis of relative potencies with a quantitative 'trustworthiness' metric provided a high quality and insightful ranking. The 'trustworthiness' score is a data quality score weighted by the maximum percentage of the product known (i.e., the best case confidence for a given product). There were several key takeaways from the comparison of multiple hazard ranking strategies. First, simple approaches such as weighted average or scaling are valuable for their accessible interpretation but are likely not protective in the sense that a relative potency approach is based on standardization against the most hazardous constituent. Second, relative potency of each specific constituent can also be calculated by chemical type of constituents—which speaks to commonalities across products both in constituents and hazards. Third, multivariate methods retain insight into endpoints that are influential in ranking or clustering among AFFF products. Fourth, incorporation of a 'trustworthiness' score provides a numerical value for decision makers on the confidence that can be placed in reported rankings. The 'trustworthiness' score aids to capture uncertainty associated with the constituents and product composition, and gives a sense of the confidence associated with each formulation's rank. The score was an index of proportion experimental data (vs. predicted or read-across), completeness of data across endpoints, and an identification rate (proportion of identifiable constituents). In the end, our ranking of fluorine-free AFFF products connects meaningful endpoints and semi-quantified uncertainty analysis. Inclusion of influential data and sources of uncertainty allows for robust hazard ranking interpretation in a data-poor environment.

03.02.02 Comparative Assessment of Toxicity and Bioaccumulation of Fluorine-Free Formulations in Terrestrial Plants and Soil Invertebrates

X. Wu, Lawrence Berkeley National Laboratory / Ecology; X. Yu, D. Kim, J. Ceja-Navarro, Lawrence Berkeley National Laboratory; H. Peng, University of Toronto / Department of Chemistry

Since 2006, use of Aqueous Film Forming Foam (AFFF) containing perfluorooctanesulfonic acid and perfluorooctanoic acid by U.S. DoD has generally been replaced by short-chain AFFF foams as they are thought to be less toxic and bioaccumulative. Also, a number of alternative fluorine-free surfactant formulations are currently under development by DoD SERDP, and commercially-available fluorine-free foams are being tested under DoD ESTCP to evaluate their ability to meet current DoD performance requirements. So far, the ecotoxicity, particularly with regard to chronic toxicity of the short-chain AFFF foams and the new fluorine-free formulations are unclear. In this study, we performed both acute and chronic toxicity tests of six fluorine-free formulations (ECOPOL, ENVIRO, Avio, RF3, NFD 20-391, and NRL 502W) and one reference short-chain fluorinated AFFF (Platinum C6) in terrestrial plants and soil invertebrates using SSL soil collected from a DoD field site. One environmentally-relevant plant species, the field mustard (*Brassica rapa*), and two model invertebrates representing lower trophic levels of soil food web, the nematode *Caenorhabditis elegans* and the collembola *Folsomia candida*, were used as the test organisms. The chronic toxicity endpoints included the growth of plants and production of seeds, and the growth and reproduction of invertebrates. Moreover, to investigate the bioaccumulation potential of individual chemicals within the formulations, we developed non-targeted analysis methods using ultrahigh-resolution mass spectrometry (Orbitrap LC-MS) to determine biota-to-soil accumulation of individual chemicals in each formulation. Results to date show that the Platinum C6 is the most acutely toxic formulation among the seven test formulations to the survival of nematode *C. elegans*, while the NRL

502W is the least toxic. On the contrary, the Platinum C6 exhibited little chronic toxicity to the growth of the plant *B. rapa* while the NRL 502W significantly inhibited the plant growth. This indicates the variation of toxic effects of formulations across species. Chronic toxicity tests of formulations in *C. elegans* and *F. candida*, and investigation of bioaccumulation of formulations are still ongoing. Results from this study will assist DoD in selecting appropriate formulations that meet both requirements for fire-suppression and environmental protection.

03.02.03 Toxicity of Fluorine-Free Surfactant Formulations for Soil Invertebrates Folsomia candida and Enchytraeus crypticus

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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 DoD performance requirements. The relative toxicities of fluorine-free AFFF-alternatives (FF-AFFF), as compared with legacy AFFF formulations are not known. We are developing ecotoxicological data for seven candidate FF-AFFF formulations and a legacy AFFF formulation by determining chronic toxicity for soil invertebrates *Folsomia candida* and *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.

03.02.04 Acute and Subacute Toxicity Tests with Six Fluorine-Free Foams

A. Narizzano, A. East, L. Holden, M.A. Bazar, M. Bohannon, C.L. Procell, M. Quinn, U.S. Army Public Health Center / Toxicology

Fluorine-free aqueous film-forming foams (AFFFs) are being developed and tested for their efficacy and potential to elicit adverse effects in animals or humans. Given the novelty of these foams and the paucity of toxicological data on them, hazard evaluation is necessary to avoid regrettable substitutions. In the present work, six separate fluorine-free foams and one C6-containing foam were administered orally to mice. In acute toxicity tests, mice were dosed once via oral gavage and observed for 14 days. No adverse clinical effects, mortality, or gross pathological observations were detected; thus, the LD50 of each formation exceeded 2000 mg/kg. In subacute toxicity tests, mice were dosed daily via oral gavage for 28 days. Bioenergetics, clinical chemistry and hematology, gross pathology, and hormone analysis were used to rank the order of relative toxicity of each foam formulation. Tests will contribute to a basic toxicological understanding of each formulation and will help refine doses for combined repeated dose toxicity studies and reproduction/developmental toxicity screening tests. Ultimately, data will be incorporated into a Toxicity Assessment as a baseline to enable data-driven decisions for adoption of fluorine-free AFFF at Department of Defense (DoD) installations and the broader community.

03.02.05 Toxicity Assessment of Fluorine-Free Aqueous Film-Forming Foams

L. Holden, A. East, A. Narizzano, C.L. Procell, M. Quinn, U.S. Army Public Health Center / Toxicology

Legacy aqueous film forming foams (AFFF) contain per- and polyfluoroalkyl substances (PFAS), some of which are known to be persistent in the environment and bioaccumulative. With the urgency to replace fluorine-containing AFFF, proactive toxicology assessments of candidate replacement formulations aim to avoid regrettable substitutions. Manufacturer-supplied safety data sheets (SDS) are a reasonable first-pass assessment tool but rarely disclose the primary data used to classify the toxicity of each component. To address this issue, each assessed AFFF formulation was broken down into its component parts, and a comprehensive literature review was performed for each ingredient. Data from available literature were compiled and summarized with integrated supporting quantitative structure activity relationship (QSAR) modeling and select *in vitro* data—aquatic toxicity (i.e., bacterial, *Daphnia*), genotoxicity, dermal irritation—to provide decision makers with hazard knowledge to support data-driven decisions for adoption of fluorine-free AFFF. Individual chemical and class-based toxicity profiles were completed for each formulation and translated into a summary assessment of the toxicity for each mixture. Our goal was to (1) assess key toxicity characteristics of six fluorine-free AFFF formulations by using peer-reviewed data, government reports, and QSAR modeling, (2) highlight the information gaps that remain, and (3) recommend a path forward to address remaining information gaps. For the assessed formulations, we found that the major areas of concern for potential human health toxicity are acute ocular and dermal irritation, whereas the major environmental and ecological toxicity concerns are impacts on aquatic systems. Accurate toxicity assessment was hindered by the unreported percentages of certain components within each formulation (manufacturer-disclosed components of each AFFF ranged from 5–74.5%). Based on our toxicity assessment, concentrated forms of the fluorine-free AFFF formulations examined are likely to be acutely toxic to aquatic environmental receptors. When used at the intended dilute concentration (generally 3%), these formulations are less likely to be acutely toxic; while mobile in soil and water, they are generally not expected to be persistent in the environment. Recommendations for future testing include expanded environmental partitioning and biodegradation modeling, chronic exposure assessments in aquatic receptors, and terrestrial toxicity testing.

03.02.06 Toxicity of Per- and Polyfluoroalkyl (PFAS) PFAS-Free Aqueous Film Forming Foam Formulations to Estuarine Organisms

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Understanding the environmental hazards of potential PFAS containing Aqueous Film Forming Foams (AFFFs) is a critical need in environmental risk assessment. As PFAS-free AFFF formulations are considered for use, building the basic toxicological thresholds in estuarine and freshwater organisms will help to guide decisions and minimize risk as PFAS containing AFFFs are phased out and new fire suppression products become available for use. This study aims to develop toxicity thresholds for a series of estuarine species including mysids (*Americamysis bahia*), copepod (*Acartia tonsa*), sheepshead minnows (*Cyprinodon variegatus*) and eastern mud snails (*Tritia obsoleta*) using six PFAS-free products and one PFAS product. Definitive 48-h acute testing with larval *C. variegatus* resulted in LC50 estimates of between 5.93 and 126 mg/L for these seven AFFFs. Preliminary 48-h LC50 estimates for the larval snail *T. obsoleta* (~1-10 ug/L) suggest they are more sensitive to AFFFs than the sheepshead minnows. Acute testing is underway for the mysids and copepods, followed by chronic testing and sublethal biomarker analysis.

03.02.07 Evaluating Freshwater Toxicity of PFASs and PFAS Mixtures Associated with AFFF

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Per- or poly-fluorinated alkyl substances (PFASs) are often present in AFFF-affected sites as complex mixtures. Rigorous assessment of the ecological risks of AFFF-associated mixtures requires understanding of both the potency of individual constituents and the nature of toxic interactions (i.e. additivity, independence, synergy or antagonism) among these constituents. Our team has conducted a suite of invertebrate toxicity studies with PFASs that are most frequently detected in AFFF-affected waters. Our present work includes the 4-8 chain-length perfluorinated sulfonates and carboxylic acids, with others (e.g. fluorotelomers) planned for the near future. A focus mainly on sublethal endpoints reflects the demonstrated greater sensitivity of growth and reproduction compared with short-term lethality. Three invertebrate species (*Ceriodaphnia dubia*, *Chironomus dilutus*, and *Hyalella azteca*) were chosen to expand the taxonomic range already present in the literature. These species are also amenable to testing of growth or reproductive performance in short term tests (7 days) with large numbers of both individual PFAS and binary mixtures thereof. Of the tests completed thus far, most sublethal effect concentrations have been in the mg/L range. A notable exception is PFOS effects on *Chironomus* growth, which were in the µg/L range, consistent with reports in the recent literature. Preliminary findings that these invertebrates are generally less sensitive to shorter-chain PFASs (i.e. 4-6 carbon) compared with PFOS suggest that newer fluorinated firefighting formulations composed of short-chain alternatives would carry a reduced ecological risk compared with legacy formulations. Binary mixture studies have been completed only with *Ceriodaphnia* thus far, but these have indicated concentration additivity among and between the sulfonate and carboxylic acid PFASs tested, implying a common toxic mechanism for *Ceriodaphnia*. Additional mixture work is planned to facilitate grouping PFASs by common toxic mechanism and assessing ecological risks of AFFF-associated mixtures. 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.

03.02.08 Effects of 6: 2 FTS on Reproductive and Immune Endpoints and Determination of a Benchmark Dose in White-Footed Mice

M. Bohannon, A. Narizzano, M. Quinn, U.S. Army Public Health Center / Toxicology

The 6:2 fluorotelomer sulfonate (6:2 FTS) is a polyfluoroalkyl substance that is part of the larger group of per- and polyfluoroalkyl substances (PFAS). 6:2 FTS differs from the widely studied and distributed perfluorooctane sulfonic acid only in that the two carbon atoms closest to the sulfonate functional group are not fluorinated. Reproductive toxicity of 6:2 FTS was assessed in white-footed mice (*Peromyscus leucopus*) by daily exposure via oral gavage for 28 days pre-mating followed by 84 days in 1:1 male:female pairings for the remainder of the study. Animals were exposed to 0, 0.2, 1, 5, or 25 mg 6:2 FTS/kg-day. Pups were exposed via gestation and postnatal nursing. Litters were maintained for 10 postnatal days to assess health and viability. Parental generation animals were euthanized after 112 days of exposure. Exposure to 6:2 FTS did not cause a decrease in fertility or pregnancy, nor did it affect litter weight. Litter size and litter loss were unaffected by 6:2 FTS exposure. Spleen weight increased in males exposed to 5 mg/kg-day 6:2 FTS compared to controls and 0.2 mg/kg-day 6:2 FTS, however all other organ weights were unaffected in both sexes with exposure to 6:2 FTS. Sperm count did not change with exposure to 6:2 FTS. Levels of sex steroid hormones estrogen, progesterone, and testosterone as well as levels of thyroid hormones triiodothyronine and thyroxine did not change with exposure to 6:2 FTS. The formation of plaque-forming cells (PFC) in spleen tissue as an immune endpoint decreased in animals exposed to 5 and 25 mg/kg-day 6:2 FTS compared to 0, 0.2, and 1 mg/kg-day. A preliminary benchmark dose and low-end benchmark dose using PFC formation were calculated to be 3.66 and 2.40, respectively. In summary, 6:2 FTS does not affect reproductive endpoints, but shows evidence of immunosupression at 5 and 25 mg/kg-d. DISTRIBUTION A. Approved for public release: distribution unlimited.

03.02.09 PFAS Pollution in Surface Water and Biota of Galveston Bay Following AFFF Use During a Petrochemical Fire (Deer Park, March 17th-20th 2019, Houston, TX)

R. Nolen, Texas A&M University / Marine Biology; D. Hala, Texas A&M University, Galveston / Marine Biology

The use of aqueous film forming foams (AFFFs) has been shown to be an important point-source release responsible for elevated PFAS levels in the aquatic environment. In this study we investigate the environmental fate of PFAS in the surface waters and resident biota (shellfish and fish) of Galveston Bay, following the use of AFFFs to extinguish the petrochemical fire (from March 17th to 20th, 2019) of oil storage tanks at the International Terminals Company (ITC) in Deer Park (Houston, TX). The fire at Deer Park is estimated to have released ~696,990 gallons of oil-contaminated water and ~1.5-5 million gallons of AFFFs into the surface waters of Galveston Bay. In this presentation we report on the levels of up to twelve EPA priority PFASs in the surface waters of the Houston Ship Channel and Galveston Bay immediately following the ITC fire at Deer Park, and then up to several months following the fire. Alongside water sampling, shellfish and fish samples were also collected from various sites across Galveston Bay. The biota sampled included commercially important fish and shellfish species comprising: red drum (Sciaenops ocellatus), gafftopsail catfish (Bagre marinus), spotted seatrout (Cynoscion nebulosus), and the eastern oyster (Crassostrea virginica). PFAS body-burden was quantified in the muscle (edible portion) and liver of fish, or gill/mantle homogenates for oysters. Therefore, this presentation will report on the environmental fate following the acute release of PFASs into Galveston Bay. Furthermore, the measured body-burdens of PFAS will be compared with established regulatory limits for seafood safety.

03.02.10 Relative Toxicity and Bioaccumulation of Short-Chain Perfluoroalkyl Sulfonic Acids in the Hermaphrodite Freshwater Snail Planorbella pilsbryi (Gastropoda Planorbidae)

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Per- and polyfluoroalkyl substances are high-production usage chemicals used as ingredients or intermediates of surfactants, and as surface protectors for a variety of industrial and consumer products. Over the last few decades, perfluorooctane sulfonic acid (PFOS) has been under scrutiny due to its persistence, toxicity, potential for bioaccumulation and global distribution. Though trends for decreasing concentrations of PFAS have been reported in aquatic biota, this may be at the expense of shorter-chain replacement products (C6 and C4 sulfonic acids), presumed to exhibit lower toxicity and bioaccumulation potential. Yet, few studies have validated this prediction. In the present study, we assessed the toxicity of two short-chain perfluoroalkyl sulfonic acids in adult and juvenile freshwater snails (*Planorbella pilsbryi*), using a 7-day assay examining survival, behaviour, and egg production (adult snails), and survival and growth (juveniles). In adult snails, subchronic values (the geometric mean of the

NOEC and LOEC) of 22, 224 and >500 mg/L were calculated for PFOS (C8), perfluorohexane sulfonic acid (PFHxS - C6) and perfluorobutane sulfonic acid (PFBS - C4), respectively, confirming that toxicity increased with chain length. In juveniles, subchronic values were 71 mg/L (PFOS), >500 mg/L (PFHxS), and >500 mg/L (PFBS). Reproductive output in adult snails was the most sensitive endpoint investigated, suggesting that earlier life stages were not more sensitive than adults. Further research is in progress to assess the relative toxicity of perfluorooctane carboxylic acid (PFOA - C8), perfluorohexane carboxylic acid (PFHxA – C6) and perfluorobutane carboxylic acid (PFBA - C4). Tissues of adult snails have been preserved to assess bioconcentration, for which analyses are pending.

03.02.11 Perfluorobutanoic Acid (PFBA) Exposure Induce Non-Monotonic Development Perturbation in Soybean and Cherry Tomato Plants

E. Omagamre, University of Maryland Eastern Shore / Natural Sciences; F. Ojo, S. Zebelo, University of Maryland Eastern Shore / Department of Agricultural and Food Sciences; J. Pitula, University of Maryland Eastern Shore / Department of Natural Sciences

Studies on the phytotoxic impacts of PFAS are emerging. Most of these studies however involve legacy PFAS such as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) and are mostly carried out at concentrations higher than observable in the environment. In this study, development indices of soybean (*Glycine max* L.) and cherry tomato (*Solanum lycopersicum var. cerasiforme*) plants grown with irrigation water containing environmentally relevant levels of perfluorobutanoic acid (PFBA) were evaluated. Soybean and cherry tomato seeds were surface sterilized and sown in polypropylene pots containing 70 g of wet sterilized soil. Irrigation of the sown seeds as 7-treatment groups with PFBA-spiked solution at concentrations ranging from 0.1 ng/L to 1 mg/L was commenced. Impact evaluation was carried out on the soybean plants after growing for 4 weeks while the tomato plants were allowed to grow until they bore fruits. Obtained data suggests a non-monotonic impact of PFBA on chlorophyll content, growth height, flowering and fruit bearing. The 100 ng/L treatment group showed the most developmental stimulation of the treatment groups, with 117%, 128%, and 150% stimulation over the controls respectively for leaf chlorophyll content, plant height and total number of produced fruits. Groups treated with less than 100 ng/L PFBA generally produced less flowers and fruits compared to the controls. Plant flowering data suggests that the 1 mg/L group were stressed as they produced a significantly higher number of flowers than the controls and the other treatment groups but also failed to develop fruits. The overall data suggests that PFBA modulated some growth and development pathways in the studied plants at environmentally relevant exposure levels.

03.02.12 Toxicity of Non-Fluorinated Fire Fighting Foams to Northern Bobwhite Quail (Colinus virginianus)

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Concerns about possible adverse environmental and human health impacts of long chain PFAS has spurred the development of non-fluorinated fire-fighting foams. It is important to also determine the potential ecotoxicity of these non-fluorinated foam products. We conducted an acute oral toxicity test in Northern Bobwhite quail (*Colinus virginianus*) using an up-and-down procedure with six different fluorine-free foams. A commercial short-chain fluorinated foam was also tested for comparative purposes. Groups of 5 birds were initially pseudo-gavaged with a volume of each product corresponding to a "limit" (the highest concentration expected to occur environmentally). Only one bird (1 of 35) died during the limit test, indicating that all 7 products have an acute LD50 in adult quail at or above the limit (~ 1500 mg/kg body weight). In preparation for a chronic study with exposure via drinking water, we also completed a water avoidance trial over 5 days using a short chain fluorinated foam on three pairs of birds at three different concentrations (0.5, 1.5 and 3%). Based on comparisons to water consumption in control birds, we selected foam concentrations for our chronic study below 0.5%. An initial chronic study was conducted with adult pairs of Northern Bobwhite quail which focused on survival, growth, and reproduction endpoints. Birds were photo-stimulated and then exposed via drinking water to either a non-fluorinated foam or a short-chain fluorinated foam for 60 days. Water consumption was used to determine average daily intake. Adults were monitored for survival, growth, and reproductive output. Hatching success, chick survival, and chick growth (21-d) were also determined.

03.02.13 Toxicity of Fluorine-Free Surfactant Formulations for Three Plant Species in a Natural Soil

M. Simini, U.S. Army DEVCOM Chemical Biological Center / Molecular Toxicology Branch; R.G. Kuperman, U.S. Army Chemical Biological Cntr / Molecular Toxicology Branch; G.R. Lotufo, U.S. Army Corps of Engineers / Environmental Laboratory

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 DoD performance requirements. The relative toxicities of fluorine-free AFFF-alternatives (FF-AFFF), as compared with legacy AFFF formulations are not known. We are developing ecotoxicological data for seven candidate FF-AFFF formulations and a legacy AFFF formulation by determining chronic toxicity in soil for three plant species: *Medicago sativa* (alfalfa), *Echinochloa crus-galli* (barnyard grass), and *Lolium perenne* (perennial ryegrass). Test species were exposed to each formulation in separate range-finding studies 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.

03.02.14 Toxicity of Fluorine-Free Surfactant Formulations for the Earthworm Eisenia andrei

G.R. Lotufo, U.S. Army Corps of Engineers / Environmental Laboratory; R.G. Kuperman, U.S. Army Chemical Biological Cntr / Molecular Toxicology Branch; M. Simini, U.S. Army DEVCOM Chemical Biological Center / Molecular Toxicology Branch; R.E. Boyd, P. Krupa, M. Jung, US 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 DoD performance requirements. The relative toxicities of fluorine-free AFFF-alternatives (FF-AFFF), as compared with legacy AFFF formulations are not known. We are developing ecotoxicological data for seven candidate FF-AFFF formulations and a legacy AFFF formulation by determining chronic toxicity for the earthworm *Eisenia andrei* exposed 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.

03.02.15 Comparative Assessment of Acute and Chronic Toxicity of Fluorine-Free Formulations in Caenorhabditis elegans

X. Yu, J. Ceja-Navarro, X. Wu, Lawrence Berkeley National Laboratory

Aqueous film-forming foam (AFFF), a highly efficient firefighting foam that extinguishes flammable liquid fires, has been found to present human health risks and environmental concerns due to their persistence in the environment as well as high bioaccumulation potential. Currently, fluorine-free firefighting foams are considered one of the most promising candidates for AFFF-alternatives, however, the potential environmental risk and ecotoxicity of them have not been well studied. This research aims to investigate the ecotoxicity assessments of six fluorine-free formulations, including Ecopol, Enviro, Avio, RF3, NRL 502W, NFD 20-391, as well as one reference short-chain AFFF formulation Platinum C6 on nematode *Caenorhabditis elegans* in SSL soil collected from a DoD field site. We performed acute (24 h) and chronic (96 h) toxicity tests to estimate LC_{50} values for survival and EC_{50} values for the growth and reproduction of *C. elegans*. Results to date show that Platinum C6 is the most acutely toxic formulation among the seven test formulations, while the NRL 502W is the least toxic formulation to the survival of *C. elegans*. This study's findings will assist DoD in selecting appropriate firefighting foams that meet both requirements for fire suppression and environmental protection.

03.02.16 Acute Toxicity of Fluorine-Free Aqueous Film Forming Foam Alternatives for Eight Species of Larval Amphibians

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Per- and polyfluoroalkyl substances (PFAS) are contaminants 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 (AFFF). Over the last several years, many fluorine-free AFFF alternatives have been developed that include diverse technologies such as siloxanes, biopolymers, nanoparticles, and ionic liquids. While research addressing the suitability of these alternative technologies as replacements for PFAS-based AFFF is ongoing, we also need research that addresses their potential environmental impacts prior to their use. Aquatic habitats are critical for ecological risk characterization of fluorine-free AFFF alternatives because these systems are often highly valued by stakeholders and regulators. Amphibians are dependent on aquatic habitats for both adult and larval life stages, increasing the likelihood that they will be exposed to fluorine-free AFFF compounds released in these systems. The objective of this study was to evaluate the acute toxicity of six fluorine-free AFFF formulations in a variety of amphibian species and compare toxicities to a PFAS-containing AFFF formulation (Buckeye Platinum). We conducted LC50 tests with eight larval amphibian species: Jefferson salamanders (*Ambystoma jeffersonianum)*, American toads (*Anaxyrus americanus*), western chorus frogs (*Pseudacris triseriata*), gray treefrogs (*Hyla versicolor*), wood frogs (*Rana sylvatica*), northern leopard frogs (*R. pipiens*), green frogs (*R. clamitans*), and American bullfrogs (*R. catesbeiana*). Among all species tested to date, Avio Green and Buckeye Platinum are the most ($LC50_{48hr}$ 33.2 – 61.5 mg/L) and least ($LC50_{48hr}$ 1409.9 – 2012.5 mg/L) toxic formulated products, respectively. In addition, according to the USEPA's ecotoxicity categories for aquatic organisms, the other five fluorine-free AFFF products are 'practically nontoxic' ($LC50_{48hr} > 100$ mg/L) for the amphibian species tested. In our presentation, we will report 48- and 96-hr LC50 toxicity values for all product formulations in all eight amphibian species. Our study is the first to report species- and compound-specific differences in sensitivity to fluorine-free AFFF compounds across a wide range of amphibians. These preliminary data will be useful for informing decisions regarding the use of these compounds in areas associated with aquatic habitats.

03.02.17 The Impact of Select Per- and Polyfluoroalkyl Substance-Free Aqueous Film-Forming Foams on the Eastern Oyster, Crassostrea virginica

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Aqueous film forming foams (AFFFs) are an important tool in fire suppression used in the aviation and defense industries. These formulations historically contain 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, PFAS-free AFFFs are being examined and this study aims to evaluate the potential toxicity of a series of PFAS-Free AFFFs to the eastern oyster, *Crassostrea virginica*. Toxicity thresholds evaluating the impact on oyster embryo-larval development, larval settlement, adult shell repair, and adult oxygen uptake rates will all be conducted and compared to a current use AFFF. These data are tied to a larger effort linking testing results from NOAA, NIST and the US ACE/ERDC. The results of these tests, when combined with data from other marine and freshwater species, are expected to be used to help inform decisions surrounding the selection of replacement PFAS-free AFFFs.

03.02.18 An Evaluation of Aquatic Receptor Sensitivities to Novel Fluorine-Free Firefighting Foam Versus Short Chain PFAS AFFF Products

J.G. Suski, M. Hudson, EA Engineering, Science, and Technology, Inc., PBC / Water and Natural Resources; M.K. Chanov, EA Engineering, Science, and Technology, Inc., PBC / Ecotoxicology; C. Salice, Towson University / Environmental Science & Studies Biology; J. Guelfo, Texas Tech University / Civil, Environmental, and Construction Engineering; T.A. Anderson, Texas Tech University / Environmental Toxicology

Given the high-profile nature of the legacy contaminants resulting from wide-spread use of aqueous film forming foam (AFFF) by the Department of Defense (DoD) and in industrial and commercial practices there is an imperative need to characterize environmental concerns on the proposed replacement products for fire suppression needs. The overarching goal of this research is 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 candidate AFFF-alternatives [fluorine-free (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. The focus of this presentation is on the aquatic research element of the project. Acute and chronic toxicity tests were performed on green algae (*Raphidocelis subcapitata*), invertebrates (Midge, *Chironomus tentans*), and fish (Fathead Minnow, *Pimephales promelas*). Range finding data (LC50) for the FF products for algal toxicity are 0.72 – 192.1 mg/L and the comparative short-chain PFAS AFFF was near the mid-point within this range. The range finding data (LC50) for the FF products for chironomids are 3.08 – 533 mg/L and the comparative short-chain PFAS AFFF was near the lower end of this range in toxicity. Finally, range finding data (LC50) for the FF products for fathead minnows are 3.64 – 59.6 mg/L and the short-chain PFAS AFFF was outside of this range with a LC50 of 944.6 mg/L. Analysis of preliminary data suggests algae may be most sensitive to some FF foam products; however, fish appear to have a narrow range of sensitivity to FF products and may also experience toxicity at lower concentrations compared to short-chain PFAS AFFF. These data are undergoing further synthesis and comparison while definitive aquatic toxicity testing is expected to be completed in the near term.

03.03 Ecotoxicology and Risk Assessment of Reptiles and Amphibians

03.03.01 Effects of Selenium Exposure on the Hepatic Transcriptome of Yellow-Bellied Sliders (Trachemys Scripta Scripta)

D.L. Haskins, Purdue University / Department of Forestry and Natural Resources; M.D. Hale, University of Georgia / Ecology; S.L. Bock, B.B. Parrott, University of Georgia / Odum School of Ecology; T.C. Glenn, University of Georgia / Department of Environmental Health Sciences; T.D. Tuberville, University of Georgia / Savannah River Ecology Laboratory

Selenium (Se) is an essential element that is important for many biological functions. Exposure to excess Se can also lead to toxic responses in vertebrates. Several studies demonstrate that reptiles may be exposed to significant levels of Se, however little is known regarding toxicological effects of exposure in this group. In light of this, we sought to elucidate effects of Se in a chelonian model species, *Trachemys scripta scripta*. We exposed 70 juvenile *T. scripta* to one of three seleno-L-methionine (SeMet) treatments (control, n = 24; 15 mg/kg, n = 23; and 30 mg/kg, n = 23) via oral gavage for 5 weeks. Exposure to the highest SeMet treatment caused negative hematological outcomes (lymphopenia, heterophilia, and hemolytic anemia), histological abnormalities in claw and kidney tissues, and mortality. We also conducted RNA-seq on hepatic tissue from 24 slider turtles (n = 8 per treatment) to characterize transcriptomic impacts of Se; we observed substantial responses in exposed animals relative to controls. High dose (30 mg/kg) and low dose (15 mg/kg) Se exposure induced 644 and 88 significant differentially expressed genes (DEGs), respectively. Functional analysis (gene ontology: biological process) of DEGs suppressed by high dose Se exposure revealed consistent enrichment of immune pathways while upregulated genes were enriched for pathways related to cell proliferation. To further elucidate how exposure to Se might structure hepatic transcriptional patterns, we conducted network analysis via WGCNA (weighted correlation network analysis). This approach constructed a hepatic transcriptional network comprised of 26 highly correlated clusters, or modules, of co-expressed genes. Se was significantly associated with six of these modules, suggesting that exposure plays a substantial role in shaping the hepatic transcriptome at large. Pathway analysis (GO:BP) of Se-associated modules generally corroborated findings from targeted DEG analyses: modules negatively associated with Se were enriched for pathways related to immune regulation and activation of various leukocytes, while positively-associated modules were enriched for pathways related to mitochondrial organization and transport. Collectively, our study demonstrates that *T. scripta* (1) exhibit diverse physiological and transcriptomic responses to Se, (2) effects of exposure likely span multiple tissues and physiological processes, and (3) immune function might be particularly responsive.

03.03.02 Oil Dispersant Component Dioctyl Sodium Sulfosuccinate Alters Thyroid Hormone Signaling in American Bullfrog Tadpoles

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Dioctyl sodium sulfosuccinate (DOSS) is a constituent of Corexit 9527 and 9500A, laxatives, and numerous pharmaceuticals, cosmetics, and food products, and is known to disrupt adipocyte differentiation signaling by targeting nuclear receptor-mediated gene expression through peroxisome proliferator-activated receptor gamma (PPARg). Hormone signaling through related receptors is accomplished by the thyroid hormones (THs), thyroxine (T4) and triiodothyronine (T3) to regulate growth, development, and metabolism in vertebrates. The present study aimed to determine if DOSS was capable of disrupting TH signaling using the American bullfrog, *Rana (Lithobates) catesbeiana*, tadpoles that underwent TH-dependent metamorphosis to a terrestrial juvenile frog. Premetamorphic *R. catesbeiana* tadpoles were injected with 2 pmol/g body weight T3 or 10 pmol/g body weight T4 to induce precocious metamorphosis, then exposed for 48 hours to 0.5, 5, and 50 mg/L DOSS. Gene expression of three classical TH-responsive targets (*thra*, *thrb*, and *thibz*) was measured in tadpole liver and tail fin tissue through reverse transcription quantitative polymerase chain reaction (RT-qPCR). Exposure conditions that elicited a significant gene expression response were evaluated using RNA-seq. DOSS disrupted gene expression in liver and tail fin tissue at all three concentrations tested, but the patterns of expression varied in tissue, gene transcript, and TH treatment status. To our knowledge, the current study is the first demonstration that DOSS can alter TH signaling. Further exploration into DOSS disruption of TH signaling is warranted as exposure may affect other TH-dependent processes such as salmon smoltification, flatfish metamorphosis, bird molting, and perinatal human development.

03.03.03 Toxicological Effects of Organic Contaminants in Hawksbill Sea Turtle Skin Cell Cultures

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Sea turtles face a number of anthropogenic and natural threats. The hawksbill sea turtle is listed as critically endangered according to the IUCN red list and can be found in all warm oceans. *In vitro* toxicology provides a crucial means to investigate the impact of pollutants in this species for which *in vivo* experiments are prohibited. Fibroblast cultures were successfully established from skin biopsies obtained from ten healthy hawksbill sea turtles rehabilitated at the NOAA/NMFS Sea Turtle Hospital in Galveston, Texas. This project examined the cytotoxicity of crude oil, Corexit™9500A, perfluorooctanoic acid (PFOA), polychlorinated biphenyl (PCB77), and benzo[a]pyrene (B[a]P) via both MTT (methylthiazolyldiphenyl-tetrazolium bromide) at 24h and 96h and LDH (Lactate Dehydrogenase) assays at 24h. In the crude oil and oil dispersant study, cells were dosed with either media accommodated fractions of crude oil and/or Corexit™9500A. Results reveal significant toxicity compared to the control after exposure to Corexit after both timepoints. Exposure to Corexit for 96h resulted in only 1% cell viability while 24h exposure resulted in 3% cell viability. In the PFOA study, cells were dosed with concentrations of 0.05 µM, 0.5 µM, 5 µM, 50 µM and 500 µM. Results indicate significant toxicity compared to the control after exposure to the 5 mM and 500µM concentration after both timepoints, and 5µM concentration for 96h. Exposure to the 500µM concentration for 24h resulted in 57% cell viability. In the PCB77 study, cells were dosed with concentrations of 0.01µM, 0.1µM, 1.0µM, or 10µM. Results reveal significant toxicity compared to the control after 96h exposure to 0.1, 1.0 and 10µM doses. Exposure to the 10µM concentration for 96h resulted in 71% cell viability. In the B[a]P study, cells were dosed with concentrations of 0.01µM, 0.1µM, 1.0µM, or 10µM. Results indicate significant toxicity compared to the control after exposure to 0.1µM, the 1.0µM and 10µM doses for 96h. Exposure to the 10µM concentration for 96h resulted in 71% cell viability. The 24h and 96h LC50 values will be calculated for PFOA, PCB77 and B[a]P to determine precise concentrations to be used for toxicity comparisons. Cytotoxicity observed via MTT and LDH assays will be compared to (1) assess which assay may be more suitable for each contaminant and (2) determine the relative sensitivity of Hawksbill skin cells to the panel of contaminants tested.

03.03.05 The Relative Sensitivity of a Tropical Poison Arrow Frog (Epipedobates anthonyi) Compared to a Temperate North American Species (Lithobates sphenocephalus)

S.M. Weir, H. Frey, Queens University of Charlotte

Global amphibian declines are focused in tropical regions, but most of the ecotoxicological data on amphibians is collected on temperate northern hemisphere species of anurans. Others have argued that we may be missing more sensitive species because we are not regularly testing these tropical species for their sensitivity. We tested the hypothesis that tropical anuran larvae from Anthony's poison arrow frog (*Epipedobates anthonyi*) would be more sensitive than a north American native species (southern leopard frog, *Lithobates sphenocephalus*) to a variety of pesticides. We first assessed whether the pesticides appeared to be toxic below 100 mg/L using a range finding test with 1, 10, and 100 mg/L. For those pesticides with apparent toxicity at or below 100 mg/L, we exposed both species in the larval stage to the same 4 concentrations for multiple pesticides and measured time-to-death as our endpoint. We used time-to-event analysis to statistically compare sensitivity of the two species and estimated traditional LC50s for comparisons when possible. So far, we have assessed 6 pesticides and only 2 have shown toxicity below our cutoff of 100 mg/L. Data collection is ongoing for other pesticides. Results from traditional LC50s do not show significant differences in sensitivity to the two tested pesticides. However, when using time-to-event analysis, we got a more mixed result. Anthony's poison arrow frog was more sensitive to 27 mg/L pendimethalin compared to southern leopard frogs. For terbufos, at 9 mg/L southern leopard frogs responded significantly quicker than the poison arrow frog. While results are certainly preliminary, it appears that testing temperate species does not necessarily bias the researcher by using more tolerant taxonomic groups. Our data also support the continued use of fish as surrogates for amphibian larvae as none of the species tested were more sensitive than the most sensitive fish toxicity value available.

03.03.06 Internal Distribution and Maternal Transfer of Emerging and Legacy Persistent Organic Pollutants in Sea Turtles: A Meta-Analysis

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Knowledge regarding internal distribution and subsequent maternal transfer of persistent organic pollutants (POPs) within organisms is of critical importance to interpret tissue-specific biomonitoring results and refine risk assessments. We aimed to identify the factors driving tissue distribution and maternal transfer of POPs, and to identify challenges and opportunities to synthetize our current knowledge on this topic. We systematically reviewed 40 years of data on POPs in sea turtle tissues across all species. We developed a lipid database allowing harmonization of data on POP concentrations on a lipid basis. Subsequently, we assessed whether the distribution of lipid normalized POP concentrations is equal among tissues as expected by lipid based tissue affinities under steady state. Our results supported equal partitioning among tissues with high blood flow or perfusion including heart, kidney, muscle, and lung. However, observed differences in brain, fat, and blood plasma suggested the importance of physiological features including the blood-brain barrier, blood perfusion, and protein content. Differences in the distribution of legacy and emerging POPs could be explained by observed differences between species, life stages, and sexes. Species-specific differences related mainly to energy storage, while intraspecific differences were most notable between juvenile and adult stages. Additionally, our results suggested a selective maternal transfer of individual compounds. This selective transfer was likely influenced by biological factors such as the foraging and remigration behaviour and its interaction with the vitellogenesis process, and chemical characteristics such as diffusion limitation and selective release and reabsorption. Challenges in the synthesis of current data on POPs across different tissues relate to a number of ATTAC issues, specifically: data Access, Transferability and TrAansparency among studies with different methods and analytical approaches, and wise use of Conservation-sensitive materials. To increase opportunities in the use of current data for future research, we propose best practice guidelines for these ATTAC issues. The comprehensive tissue partitioning and maternal transfer patterns presented here provide a quantitative basis to support comparative assessments of POP pollution derived from biomonitoring among multiple tissues, and assist in refining toxicity assessment with tissue-specific concentration data.

03.03.07 Informing the Future: A Regulator's Perspective on Opportunities to Advance Pesticide Assessment for Herpetofauna

C. Aubee, U.S. Environmental Protection Agency / Office of Pesticide Programs

In recent years, significant energy has been invested to characterize both achievements and gaps in ecotoxicology and risk assessment of herpetofauna. While surrogate approaches in pesticide risk assessment are well established, differences in life history, exposure potential, and chemical sensitivity of reptiles and amphibians are known to produce variability in actual risk to these diverse species. As regulatory agencies move away from animal testing and toward New Approach Methods (NAMs), researchers might consider how data obtained through traditional research methods may be used to define and parameterize NAMs that advance the assessment of herpetofauna. Where data are sufficient, retrospective analyses may be particularly beneficial in guiding these future efforts. Disclaimer: This abstract reflects the views of the author and does not necessarily represent the position or policies of USEPA.

03.03.08 Quantifying Harm to Sea Turtles From Ingested Plastic: Review of Literature and Guidelines for Reporting Evidence of Harm

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More than 8 million metric tons of plastic are discharged into the oceans per year, creating a global conservation concern. Plastic ingestion has been identified as a threat to marine megafauna worldwide, including sea turtles. Lethal effects from the ingestion of plastic include obstruction, perforation, torsion, and linear erosion of the gastrointestinal tract. However, attributing mortality of sea turtles to plastic ingestion is difficult and the magnitude of its impact is debated. Unavoidable biases in sampling sea turtles, non standardized methods for inspection of pathology, and a spectrum in expertise by evaluators interpreting data have resulted in publications with polar opposite interpretations. The discrepancies and debates are the impetus for this review. For example, one study claims as little as 14 pieces of plastic has a 50 % probability of mortality and another suggests 0.5 g of plastic can cause mortality. However, other studies have found turtles with upwards of 50 pieces or 12 g of plastic that showed no signs of harm. Furthermore, the lack of standardization in data reporting makes it difficult to compare among studies. The objectives of this study were to 1) review the literature on plastic ingestion impacts in sea turtles, 2) score the quality of evidence provided by studies and inspect for potential biases, and 3) suggest reporting guidelines for showing evidence of impact. Ninety-four publications were included in this review, totaling 236 studies of individual sea turtle species. Each study was reviewed by two veterinarians, and given scores for how the evidence was presented based on eight questions: type of study, inclusion of photos displaying harm, consultation by a veterinarian, description completeness of the plastic causing harm, evidence of pathology from the plastic, description of body or post-mortem condition, evidence of gross or microscopic pathology. The studies assessed had an average score of 3.4 out of 13 possible points, and 32.6 % of them concluded that plastic ingestion caused harm. Of those, only 6 % provided evidence of the harm through photos of the harm, and only 5 % stated that a veterinarian was involved with the necropsy. More rigorous standardized evidence of harm from plastic is needed, specifically in the form of documenting pathology associated with plastic ingestion and absence of other causes of death, to definitively identify plastics as the primary cause of death.

03.03.09 Foul and Loathsome Habitats for Foul and Loathsome Creatures? Salinity Impacts on Spontaneous Swimming Behavior of Pickerel Frog Tadpoles

L. Lockett, Rutgers University / Department of Ecology Evolution and Natural Resources; L. Hazard, Montclair State University

Species of North American amphibians have been observed to utilize saline wetlands for reproductive sites year after year, with many New Jersey wetlands exceeding the Environmental Protection Agency thresholds for chronic and acute concentrations of chloride. Research has suggested that some amphibian species have evolved abilities to survive in these saline systems with varying capacities and different susceptibilities to the increase, yet the literature also has a plethora of recorded negative impacts of salinity in laboratory settings; results from laboratory work is contradictory to field observations. Background levels are typically low, but there are wetlands that can spike extremely high in the state of New Jersey, and it is unknown, how these short-term fluctuations of increased salinity impact spontaneous tadpole behavior. This study assessed the impact of increasing baseline salinity conditions on pickerel frog tadpoles collected in May 2020 in a wetland near Assunpink Wildlife Management area by exposing tadpoles to elevated sodium chloride (NaCl) concentrations for thirty-minute intervals to assess spontaneous behavior changes. ToxTrac was used to analyze the videos of the recorded trials. An analysis of covariance was run for each response variable measured for the experiment. We found that acute increases in NaCl over a thirty-minute timespan did result in changes in spontaneous behavior; specifically, there was a statistically significant effect on the proportion of the arena explored, and total distance traveled. However, there was no difference across treatment groups in the amount of time that the tadpoles spent motionless. These findings are important because they highlight the fact that tadpoles may be capable of decision making and attempt to find more desirable refuges in their natal wetland if necessary.

03.03.10 Effects of Indirect Agricultural Spray Drift on Amphibians and Their Habitats in Southern Georgia

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Pesticides are used to control insects and diseases; however, non-target exposure to amphibians and other species frequently occurs. We determined pesticide concentrations in amphibians and stemflow from two agriculturally impacted wetlands near Tifton, Georgia and Joseph Jones Ecological Research Center (JJERC), Albany, Georgia to assess the risk from spray drift contributions to non-targeted habitats. Amphibians were collected from three wetland types: reference (semi-forested interior sites not likely to be impacted by spray drift), agricultural fields, and edge (sites that border agricultural fields, and likely impacted by spray drift). All samples were analyzed for > 200 pesticides, with more than 30 pesticides detected across all matrices. The herbicide metolachlor was the most frequently detected pesticide with highest concentrations in stemflow samples. Additionally, a few legacy pesticides such as mirex and DDE were detected in amphibian tissues collected at both study sites in addition to reference, edge, and agricultural sites. Trace levels of parent pesticides were found in amphibians, likely due to hepatic metabolism. Stemflow concentrations were compared against environmental water screening values and aquatic life benchmarks as a proxy to determine hazard to amphibians. Several pesticides were present at concentrations that exceeded the aquatic life benchmark value. Exposure to pesticides through spray drift on tree trunks and resultant stemflow could be problematic for many amphibians, especially treefrogs, due to their arboreal residence times. Additionally, pesticide mixtures were present in all environmental matrices, making it difficult to determine the adverse effects these chemicals may have due to potential additive, antagonist, or synergist effects. Amphibian populations are in decline and pesticide exposure has been identified as one of the primary causative factors. These data can aid in the assessment of risk of indirect spray drift and subsequent exposures for amphibian species and their wetland habitats.

03.03.11 Toxic Effects of 3 Nonsteroidal Anti-Inflammatory Drugs (NSAIDS) on the African Clawed Frog Xenopus laevis

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NSAIDs are drugs most consumed worldwide because used to remedy the pain of mild to medium grade and they are over-the-counter. These drugs are present in hospitals effluents and has been demonstrated which are not degraded in water treatment systems for this reason have been detected in surface water at concentrations of 6.5 ng/L to 100 ug/L. Because the effects of these drugs on aquatic organisms are not known in this work, the oxidative, neurotoxic and genotoxic effects of 3 analgesics: Naproxen, Paracetamol and Acetylsalicylic Acid was evaluated in the African frog *Xenopus laevis*. Frogs specimens were obtained by donation. They were grown in the laboratory under the following conditions: Temperature 23 ° C, Photoperiod: 16 hours Light/8 hours Darkness. Water hardness: 160 mg. With juveniles of 3 cm in length, bioassays were performed where 6 organisms were exposed to a sublethal concentration of the drugs (LC1) for 15 days. Skin samples were taken for the determination of lipoperoxidation (Tbars), brain to determine the activity of the enzyme acetylcholinesterase and blood for the evaluation of micronuclei. A "t" test was carried out between the control and the exposed organisms to detect the differences in the responses to the drugs. In the results obtained, significant differences were observed between the control and the organisms exposed to NSAIDs. The analgesic with the highest oxidant effect (12.6 nM Tbars mg-1) was paracetamol (control = 0.94 nM Tbars mg-1). A decrease in AchE activity of more than 20% was observed in the Naproxen tests. The 3 drugs had genotoxic effects. The highest frequency of micronuclei was obtained in the tests with paracetamol (4.2%) and the lowest frequency in the tests with acetylsalicylic acid (2.5%). The results indicated that in sublethal concentrations tested NSAIDs have deleterious effects on frogs. It is important to study the effect of these compounds in aquatic systems, to determine the degree of risk in which organisms are chronically exposed to these drugs.

03.03.12 Hawksbill Sea Turtle (Eretmochelys imbricata) Toxicology Review

C.A. Godard, A. Csipak, Texas Tech University / Department of Environmental Toxicology

Sea turtles are long-lived protected species vulnerable to both natural and anthropogenic threats. The hawksbill sea turtle (*Eretmochelys imbricata*) was declared critically endangered in 1996 by the IUCN due to a combination of pressures including over-exploitation, habitat degradation, fisheries, pollution etc. Hawksbills have a lifespan of approximately 50-60 years, during which they can bioaccumulate and biomagnify a variety of marine contaminants. Their habitat includes all warm oceans making them vulnerable to exposure to numerous environmental contaminants. The hawksbill's diet is primarily comprised of sponges with supplementation of plants, small fish, and invertebrates. This review aims to highlight the state of toxicological knowledge regarding hawksbill sea turtles and includes 35 peer reviewed articles focused on chemical stressors. References were obtained via a thorough and multi-tiered Web of Science literature search. Three types of toxicology studies were included: those describing exposure to toxicants, those reporting on detection and quantification of specific toxicants, and those related to potential effects following exposure. The categories of toxicants so far studied in hawksbill sea turtles include but are not limited to trace elements, heavy metals, PCBs, DDTs, PAHs, PBDEs, PFCs, and crude oil. The literature includes a broad assessment including eggs, juvenile sea turtles, nesting females, and male and female hawksbills of unidentified age. The studies on animals of known sex all included females but only 23% included males. The earliest paper included was published in 1987 and reported on oil residues but 71.4% of the papers span from 2011 to 2021. The number of papers published on a specific class of contaminants are listed in decreasing order: trace elements, heavy metals, oil, PCBs, DDTs, PAHs, PFCs, and PBDEs. Of all the trace elements, cadmium was detected most frequently while thallium was reported as one of the most toxic. The majority of the studies were conducted in Mexico and Japan, making up 40% and 20% of the papers respectively. Gaining a better understanding of contaminant prevalence and impact for this species will assist in developing conservation strategies in the future. Characterizing past and current exposure levels in hawksbills also provides key indirect information on prey species and the surrounding ecosystem.

03.03.13 Atrazine and Estradiol Effects on Development of Acris Blanchardi (Blanchard's Cricket Frog) Exposed in Outdoor Enclosures

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The herbicide atrazine has been proposed as a potential endocrine disrupting compound (EDC) for amphibians. However, evidence of effect (e.g., feminization of phenotypic males) has lacked consistency across studies, treatment concentrations and species. Specifically, few studies have reported effects in species other than *Xenopus* at environmentally relevant atrazine concentrations. Using atrazine concentrations below or at those typically found in surface waters (0.5, 5.0, 50 µg/L), we exposed *Acris blanchardi* (Blanchard's cricket frog) larvae throughout development until metamorphosis. An additional 50 µg/L treatment (50+ µg/L) was utilized in which algae was supplemented to test whether atrazine indirectly reduced food sources. In addition to atrazine, experimental groups also included a negative control and two positive controls, 17β-estradiol (E2) at 2.3 and 25 µg/L. After 60 days of dosing during development and discontinuation of treatment, *A. blanchardi* metamorphs were euthanized for analysis of gross and histopathological development. Atrazine treatment did not influence body mass (BM), snout-vent length (SVL), gonad size, nor gonad development of male or female *A. blanchardi* suggesting atrazine does not affect development in this species. Females exposed to 50 µg/L atrazine and supplemented with algae, had 29% lower BM, 10% shorter, and 28% lower mean ovary area (mm^2) as compared to negative controls, suggesting algae enrichment had a significant negative effect. Estradiol positive controls (25 µg/L) resulted in significantly increased oviduct development in juvenile males. Ovary area was also significantly influenced by estradiol treatment (2.3 and 25 µg/L). Overall, E2 had much less effect than predicted based on other model species. *Acris blanchardi*, overall, was not affected by long-term developmental exposure to environmentally relevant concentrations of atrazine. However, this species also was largely insensitive to exogenous estradiol in this test system.

03.03.14 Steroid Hormones and Sex Determination in Reptiles: A Review

J. Farquharson, C.A. Godard-Codding, Texas Tech University / Department of Environmental Toxicology

Reptiles exhibit a form of environmental sex determination known as temperature-dependent sex determination (TSD). Here, 80 peer-reviewed publications relevant to sex determination in reptiles were reviewed in four sections: an overview of TSD; implications of environmental stressors on sex determination; steroid hormones and steroidogenic enzymes involved; and the accuracy and suitability of hormone analysis techniques for quantifying hormones and diagnosing sex in reptilian samples. TSD has been identified in all four reptilian orders but has been studied most in Testudines. During a reptile's egg incubation period, there is a thermo-sensitive period in which the sexes of the future hatchlings are determined by temperature; such that, specific temperature ranges produce one sex in a biased fashion, or both sexes in an even sex ratio. Early TSD studies identified temperature ranges that produce either sex, or both sexes, but eventually transitioned into research on the underlying molecular mechanisms; primarily related to the involvement of steroid hormones. Testosterone, dihydrotestosterone, and 17β-estradiol are the most studied hormones as it relates to TSD. Other hormones studied are estrone, estriol, 3α-androstanediol, 3β-androstanediol, and androsterone. Cytochrome-P450 aromatase and 5α-reductase are the most prominent steroidogenic enzymes seen throughout the TSD literature. Other enzymes studied are 16α hydroxylase, and 3α- and 3β-hydroxysteroid dehydrogenase. Environmental stressors can alter normal TSD outcomes by endocrine disruption of steroid hormone production, which may shift population sex ratios away from the norm and have a negative effect on species fecundity. For this reason, understanding the role of steroid hormones in reptiles became increasingly important, especially since various species, including all sea turtles, were classified as threatened or endangered in the United States. To investigate TSD alteration, researchers devised and employed techniques for diagnosing reptilian sex and assessing population sex ratios. Some techniques provide a definitive sex diagnosis, such as laparoscopy and histological examination of gonads, but are highly invasive or require euthanasia and thus, are not suitable for protected species. Other techniques, such as examination of external morphology and analysis of sex hormones in tissues, are less invasive but generally provide a less definitive sex diagnosis.

03.04 Employing Tox21 and Exposure21 in Invertebrate Risk Assessments

03.04.01 Early Product Development Screening Tools to Predict Non-Target Toxicity in Aquatic and Terrestrial Ecosystems

T.D. Lunsman, Corteva agriscience / Predictive Safety Center

Environmental sustainability is keystone to the development of successful new crop protection products by ensuring environmental safety, regulatory success, and sustainable solutions to farmers. There are substantial monetary investments in the research and development of crop protection products that never pass from the discovery stage to the development stage due to toxicity concerns that are not aligned with corporate environmental responsibility aspirations. Furthermore, the cost increases as timelines to market lengthen. To address this challenge, an early stage analysis to identify safe options and enable swift advancement to development is paramount. The use of New Approach Methodologies (NAMs) in early development that are predictive of long-term effects with limited animal testing and are conducted in a high throughput manner, enables the identification of safer crop protection products. The current study evaluates important ecotoxicity endpoints and the NAMs that enable the prediction of adverse effects in new crop protection products. This analysis shows the advantages of NAMs in aquatic (i.e., fish) and terrestrial toxicity (i.e., pollinator) that can report particularly sensitive endpoints to better inform the potential risk and efficiently establish downstream testing strategy.

03.04.02 Understanding Sequence Activity Relationships for Insecticidal Double-Stranded RNA and the Role of Bioinformatics to Inform Ecological Risk Assessments

S. Levine, Bayer AG - Crop Science Division / Regulatory

Over the past decade, a number of food crops utilizing RNA interference (RNAi) for quality and pesticidal traits have received regulatory approvals. The responsiveness of insects to oral delivery of insecticidal dsRNA has been shown to be dependent on dsRNA length and sequence match.

The sequence specific nature of RNAi allows targeting pest species with a high level of specificity, while mitigating risk to non-target organisms (NTOs). The application of bioinformatics can have an important role in the selection and design of the double stranded RNAs and in informing the assessment process for NTOs. When bioinformatics data for non-target arthropods are available and indicate that the minimum sequence requirements for RNAi activity are not met, then the need for toxicity testing is diminished and the likelihood of detecting adverse effects is low. However, when the minimum sequence requirements are met, the converse is not true; these data cannot be reliably used to predict the presence of RNAi activity. Nevertheless, bioinformatics can assist with the developing a hypothesis-based taxonomic approach for characterizing the spectrum of activity for pest control, understanding the relationship between taxonomic relatedness and activity, and aid in the selection of test species for NTO testing. This approach is in alignment with recommendations from a Scientific Advisory Panel, which recommended that while a bioinformatics analysis is not an absolute predictor of effects, in silico searches of published genomes could be used to perform a screening level assessment to identify potential NTOs for further evaluation based upon the presence of sequence matches. While there are currently only a limited number of published complete and partial arthropod genomes publicly available, additional genomes are being published at a rapid rate and are likely to become increasingly useful as a screening level tool for ERAs. This presentation will review what is known about the sequence activity relationship for insecticidal dsRNA and how this information can be coupled with activity spectrum bioassay data to inform ecological risk assessments for insecticidal dsRNA.

03.04.03 Application of New Approach Methods for Invertebrate Risk Assessment

C. LaLone, U.S. Environmental Protection Agency / ORD/CCTE/Great Lakes Toxicology & Ecology Division

The regulatory landscape is shifting away from whole animal testing for decision-making and exploring the utility of new approach methods (NAMs), which include computational techniques to understand chemical toxicity. Primarily these efforts have focused on reducing the use of vertebrate species in toxicity testing, however in certain contexts NAMs are equally applicable to enhancing knowledge pertaining to invertebrate risk assessment. It has been estimated that 97% of the ~1.25 million known species are invertebrates, with only a small subset of model invertebrates (e.g., daphnids, chironomids, Apis and Bombus bees) used for decision-making in regulatory toxicology. Therefore, it is important that the existing toxicity data for the few invertebrate model organisms are maximally exploited to protect the diversity of invertebrate species that are not tested, including the majority of beneficial pollinators. Fortunately, with decreased cost in sequencing genomes and increased efficiency and advocacy for generating quality sequence data for many invertebrate species there are NAMs that can be used to better understand the effects of chemicals across a broader range of species. For example, the adverse outcome pathway framework collects biological pathway knowledge from existing studies describing the causal linkages from molecular level perturbations through the various levels of biological organization to adverse outcomes at the individual or population relevant for risk assessment. The AOP framework has been applied to understand the biology relative to chemical and non-chemical stressors impacting the nicotinic acetylcholine receptor and ecdysone receptor, both key proteins involved in invertebrate physiology. Additionally, the USEPA SeqAPASS tool has been applied to describe the taxonomic domain of applicability for these AOPs, which defines how broadly key events and key event relationships may be extrapolated by evaluating protein conservation. These methods maximize the utility of existing toxicity and sequence data to understand knowledge gaps, define unique invertebrate biology that may require different testing strategies than vertebrates, and to extrapolate existing knowledge from model species to untested species of concern. Further, efforts are underway to develop invertebrate specific high-throughput transcriptomic methods that could aid in understanding the unique biological pathways impacted by chemicals in the environment. Together these NAMs provide opportunities to gain mechanistic insights for chemical risk assessment relative to invertebrates. The contents of this abstract neither constitute nor necessarily reflect USEPA policy.

03.04.04 Employing Tox21 and Exposure21 in Invertebrate Risk Assessments

N. Krishnan, R.A. Jurenka, Iowa State University / Entomology; S.P. Bradbury, Iowa State University / Natural Resource Ecology and Management

Adverse outcome pathways (AOPs) are frameworks that formalize testable hypotheses of how toxic chemicals interact with intracellular molecules to cause harmful effects in individual organisms. In insects, neonicotinoid insecticides are known to act through two AOPs. In the first AOP, which reflects the primary mode of toxic action, high doses of neonicotinoids cause neuronal paralysis and death through overstimulation of nicotinic acetylcholine receptors. In the second AOP, first reported in honey bees (*Apis mellifera*), lower doses of neonicotinoids alter the neuronal signaling pathway that is responsible for long-term memory, which leads to abnormal foraging behavior and colony failure. In this presentation, the discovery of a third AOP that results in disruption of butterfly and moth larval to pupal molting and prevents expansion of adult appendages (i.e., legs, wings, antennae, and proboscis) is described. Doses that cause this outcome are one to two orders of magnitude below those that act through the primary AOP. Experiments that provide information on the timing of key events, along with a review of the literature, indicate that neonicotinoids might be interfering with the function of crustacean cardioactive peptide neurons, which are responsible for successful pupal molt and expansion of appendages. Molecular initiating events by which neonicotinoids could be causing this AOP are proposed. Full elucidation of this AOP will provide mechanistic rationales for modeling low dose effects and assessing interspecies variability.

03.04.05 Estimating Screening-Level Risks of Insecticide Exposure to Lepidopteran Species of Conservation Concern in Agroecosystems

M.J. Hall, N. Krishnan, J.R. Coats, Iowa State University / Entomology; S.P. Bradbury, Iowa State University / Natural Resource Ecology and Management

The U.S. Fish and Wildlife Service defines ‘at-risk’ species as those that have either been petitioned for listing, proposed for listing, or assigned a candidate species status under the Endangered Species Act. In the lower 48 United States, there are currently 24 butterflies listed as endangered. Of these species, the Dakota skipper (*Hesperia dacotae*), Karner blue (*Lycaeides melissa samuelis*) Mitchell’s satyr (*Neonympha mitchellii mitchellii*), and Poweshiek skipperling (*Oarisma poweshiek*) are found in the north central states, in addition to the monarch butterfly (*Danaus plexippus*), which was listed as a candidate species in 2020. Loss of habitat and exposure to pesticides, particularly insecticides, are considered threats to population recovery for all five of these species. Given the range of these at-risk species, re-establishment of habitat in agricultural landscapes is typically identified as a primary conservation practice to support recovery. To evaluate conservation risks and benefits associated with habitat placed in close proximity to crop fields, estimates of exposure and toxicity of insecticides are needed for these lepidopteran species. Here we present preliminary screening-level risk analyses for lepidopteran species of conservation concern, based on an evaluation and integration of environmental monitoring and toxicity studies reported in the peer-reviewed literature. We interpreted the utility of existing insecticide residue data to estimate species-specific larval host plant exposure. Based on available lepidopteran toxicity data, we developed Species Sensitivity Distribution (SSD) models for topical exposures to pyrethroid and organophosphate insecticides; inadequate data were available for other classes of insecticides and dietary exposures. Using the generated SSD models with the available exposure data, we explored potential insecticide risks associated with establishing non-target lepidopteran habitat in agricultural landscapes. We also discuss the kinds of toxicity data needed to generate more models and reduce uncertainties in model predictions, and identify needs for future monitoring studies to address exposure data gaps.

03.04.06 A Semi-Field Colony Feeding Study Using the Common Eastern Bumble Bee (Bombus impatiens)

D.R. Schmehl, Bayer CropScience / Environmental Effects; L. Richardson, Stone Environmental, Inc.; A.R. Cabrera, Bayer CropScience / NA Environmental Effects and Risk Assessment; P. Jensen, Bayer CropScience LP / Residue Chemistry

Ecological risk assessment is a key component of the regulatory process required for registration of crop protection products in the US and elsewhere. The western honey bee (Apis mellifera) is the model test organism for pesticide risk assessments to pollinating bees, yet there is uncertainty around whether it is protective for other bees in all circumstances. Accordingly, efforts are underway in Europe to adapt honey bee test methodologies for two types of native bees, bumble bees and mason bees. To address the need to develop colony level methodology for bumble bees, in 2020 we conducted a semi-field colony feeding study with the common eastern bumble bee (Bombus impatiens) in agricultural landscapes in central Vermont. Adapting recently developed semi-field study protocols for honey bee colony feeding studies, we exposed commercially available bumble bee colonies to four concentrations of dimethoate insecticide (0.05, 0.19, 0.75, and 3.00 ppm) delivered via supplemental sugar solution, and compared these colonies to controls. We established 10 sites in three clusters around the margin of hayfields where pesticide usage was low and row crop agriculture accounted for no more than 20% of land use in the range of bumble bee foragers. Each treatment group was represented by one colony at each apiary. Hives were free to forage naturally, and were provisioned and inspected weekly over the course of the summer. We collected data on a range of individual- and colony-level endpoints relevant to bumble bee life history, including production of female reproductive (gyne) offspring, colony mass, foraging activity, and consumption of provisioned sugar solution. We found that dimethoate consumption exerted a concentration-dependent effect on these response variables, with the level of No Observed Adverse Effects Concentration (NOAEC) for most determined as 0.10 ppm dimethoate. Our work is novel in that it is the first fully-replicated semi-field colony feeding study to be conducted using B. impatiens, a key component of future risk assessment research for non-Apis bees in North America. We anticipate that our methods and results can guide future efforts to develop a standard test paradigm to assess risk of crop protection products to bumble bees.

03.04.07 Advancing Terrestrial Invertebrate Assessment Tools to Inform Regulatory Decisions

T.M. Steeger, U.S. Environmental Protection Agency / Office of Chemical Safety and Pollution Prevention

In 2014, the U.S. Environmental Protection Agency working in collaboration with the California Department of Pesticide Regulation and Health Canada's Pest Management Regulatory Agency finalized the *Guidance for Assessing Pesticide Risks to Bees*. The guidance identifies a tiered process for assessing risk to bees based initially on laboratory acute and chronic toxicity studies of adult and larval honey bees (*Apis mellifera*) and model-generated or default exposure values. Depending on the risk estimates and risk manager needs for further refinement, residue studies and/or colony-level studies under semi- and full-field conditions may be required. Although the suite of laboratory-based studies and subsequent residue and colony-level studies provide a relatively comprehensive set of data with which to evaluate the potential for adverse effects to bees from exposure to pesticides as a result of registered uses, these studies are resource intensive to conduct and review and appear to conflict with agency commitments to reduce reliance on whole animal testing consistent with Tox21 recommendations. There are also concerns regarding the extent to which honey bees serve as reasonable surrogates for social and solitary non-*Apis* bees. This presentation will discuss new approach methodologies to evaluate the taxonomic domain of various assays, the development of colony simulation models to reduce the need for whole colony testing, and EPA Office of Pesticide Programs' efforts to develop more predictive exposure models by leverage existing residue monitoring data. The presentation will also discuss the importance of Adverse Outcome Pathways (AOPs) as a conceptual framework for understanding and documenting the relationship between measurement endpoints collected at various levels of biological organization to assessment endpoints of regulatory interest.

03.04.08 Development and Validation of Cardiomyocytes Cell Line from the Euryhaline Fish Model Species Menidia beryllina

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Estuaries are critical ecosystems, as fish and animals rely on them for safe breeding grounds, sources of food, and migration stopovers. Pesticides, fungicides, and herbicides make their way into estuaries through improper waste management or via waterways, and have unintended side effects on wildlife. Fish are one such taxa that can be greatly affected due to agricultural runoff because pesticides and other chemicals in runoff can have detrimental effects on reproduction, growth, and swimming behavior. Fish living in brackish water may have high exposure to chemicals in runoff since they uptake more water to remain isotonic, and because chemical behavior can differ as salinity increases. Inland Silversides (*Menidia beryllina)* reside in brackish estuaries and are frequently used in chronic exposure studies, one of two marine species approved for the Environmental Protection Agency's (EPA) Whole Effluent Toxicity Testing. Marine toxicological studies are challenging because salinity alters the solubility of certain chemicals. There are few reliable benchmarks for pesticide toxicity levels in saltwater, and use of high fish numbers for exposures is discouraged, thus accurate risk assessment is difficult to achieve. Therefore, if cell lines from a marine fish such as the silverside can be created, they can be exposed to various concentrations of pesticides to identify baseline toxicity concentrations. Tissues from *M. beryllina* were cleaned of background contamination (e.g. yeast) with a brief soak in ethanol and 5% sodium hypochlorite prior to being adhered to a plate with trypsin and dispersed with a scalpel. Once adhered, the trypsin was aspirated and L-15 media + 20% FBS was applied. The cells were immortalized using a viral vector. A viral vector was used for more specificity that is not offered with spontaneous immortalization. Cell lines will be exposed to internally-determined concentrations in concomitantly-exposed larval fish and embryos. Baseline *in vivo* toxicity data will allow for more accurate *in vitro* experiments and policy decisions. This is important due to rapidly changing climates which can have adverse effects on estuaries and brackish fish species. In addition, this will lower the number of fish and costs associated with *in vitro* toxicology studies.

03.05 Integrating Emerging Science to Improve Estimates of Risk to Wildlife from Chemical Exposure

03.05.01 Wildlife Risk Assessment in the 21st Century: Integrating Advancements in Ecology, Toxicology, and Conservation

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Ecological approaches and methods to assess risks of chemicals for wildlife have generally remained unchanged for decades despite advances in ecotoxicological research methods and knowledge. A SETAC Technical Workshop on ‘Wildlife Risk Assessment in the 21st Century: Integrating Advancements in Ecology, Toxicology, and Conservation’ was organised to assess the status of the knowledge base on the topic, and to develop recommendations for enhancing the state-of-the-science and its application for wildlife risk assessment. This three-part workshop is based on a tiered approach, first webinars were organised to set the regulatory scene, based on which different working groups assessed *a priori* charge questions. Four working groups focus on each of: 1) risk assessment/problem formulation, 2) exposure assessment, 3) effect assessment, 4) risk characterization and management. Outcomes will be presented at the SETAC-NA meeting in Portland, and result in several review articles. The initial webinars provided an overview on the needs and requirements from regulatory contexts from different jurisdictions (e.g. US, Canada, EU) that drive the design of the wildlife studies to increase their impacts, and set the scene for implementation of novel scientific tools in regulatory contexts. Several overarching themes were identified which may need to be addressed to increase applicability of wildlife information in chemical risk assessment: 1) Reduce and clarify uncertainty (Weight of Evidence approaches, use of NOAEL/LOEAL/BMD approaches), 2) Add ecological relevance (development, validate, implement models/decision support systems), 3) Create simplicity, 4) Align with Regulatory frameworks (tiers), 5) Reduce and or improve animal testing protocols --> alternatives, 6) Develop read across between relevant species/sites/chemicals, 7) Define local/general protection goals. This presentation will provide an integrated overview of the results of the different working groups and provide high-level recommendations to advance the use of wildlife studies in chemical risk assessment.

03.05.02 Wildlife Toxicology Within U.S Regulatory Risk Assessment Frameworks

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Wildlife toxicology is important in the environmental management of chemicals across the regulatory environment in the United States. Varying regulations and resultant policies and guidance across the regulatory landscape evaluate ecological risk with commensurate methodologies to answer their different regulatory questions. Hence, there is not one universal U.S. risk evaluation and characterization method for all U.S. regulatory programs. The U.S. contingent of Workgroup 4 of the SETAC Wildlife toxicology risk assessment workshop, in consultation with the Steering Committee, focused the discussions of wildlife toxicology and risk to the following U.S. Environmental Protection Agency regulatory programs: Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) also referred to as “Superfund”; Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); and, Toxic Substances Control Act (TSCA) as amended in 2017 is also referred to as the “New TSCA”. These three U.S Laws evaluate risk in different ways. The intended questions addressed by ecological risk assessment within each regulation and information the assessment results bring to a final “risk management” decision, are not the same. CERCLA is an environmental risk reduction law specific to abandoned hazardous waste sites and uncontrolled release of hazardous materials that evaluates risk as acceptable versus unacceptable in the absence of remedial action. TSCA evaluates risks associated with the manufacture and use of specific chemicals and identifies risk as either “reasonable” or “unreasonable” for specific conditions of use. FIFRA provides for the regulation of pesticide distribution, sale, and use. The risk assessment decision point for FIFRA is to determine whether the pesticide “will not generally cause unreasonable adverse effects on the environment”. FIFRA assessments, in general, rely on a prescriptive required set of environmental toxicity data that form the basis of assessments. The New TSCA does not have a required set of data but does rely on data submissions from the manufacturers and EPA does have the power under TSCA to require Test Orders for further information where needed. The laws and the derived regulations on how risk assessments are conducted range from the CERCLA process where the process is defined in guidance without specific definition of tests or analysis that are required by the regulation, to more prescriptive risk assessments under FIFRA where specific testing of fungicides, insecticides, rodenticides, etc., are required and assessed prior to their general release to the U.S. environment. The presentation will discuss how the different regulatory end points; acceptable risk, unreasonable adverse effects, and unreasonable risk are evaluated, and how wildlife toxicology studies are incorporated when consistent with the laws and regulations.

03.05.04 Focusing Problem Formulation on Decision-Making to Improve Wildlife Risk Assessment

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Wildlife risk assessment, like other types of risk assessment, is a decision tool used to inform regulatory decisions. Alignment of appropriate science and analytical tools with policy and regulatory constraints is essential if wildlife risk assessment is to provide timely and relevant input to environmental decision processes. Identifying the correct policy question, its resolution and scale, and the bounds of acceptable decision uncertainty are critical. While the Problem Formulation provides the mechanism to meet these needs, it often is poorly developed and defined, diminishing risk assessment utility. In addition, jurisdictional differences occur between policies and regulations, with advances in ecology, toxicology, and exposure science being unevenly applied and integrated. Furthering jurisdictional goals using alternative methods have previously been suggested but have largely not been implemented. Here we present Problem Formulation examples and discuss issues associated with the Problem Formulation as they relate to prospective (e.g., pesticide registration, new chemicals, environmental assessment of future developments) and retrospective (e.g., contaminated site) risk assessments for wildlife. Recommendations for improving risk estimates and filling research gaps to better inform decision making are also provided.

03.05.05 Are We Getting There? What Is Broken with Methods Used to Evaluate Risk to Wildlife?

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The basic framework for ecological risk assessment (ERA), conceptually similar to the National Research Council (1983) paradigm for human health risk assessment, was formulated in 1989 for hazardous waste sites and subsequently presented as guidance in the United States in 1992 and updated in 1998. Other countries followed suit with similar guidance. The hallmark of the guidance was that it was flexible; structured to allow practitioners to adapt procedures to fit the nature of the issues at hand. Various agencies and jurisdictions modified certain features to coincide with their publics' tolerance to risk and access to data. Initially, many practitioners spoke against the flexibility in favor of more prescriptive steps. Such calls resulted in supplemental guidance being published in the US and other countries (e.g., Generic Assessment Endpoints, 2003, expanded in 2016). Within agencies, differences in methods emerged that were shaped by legislative mandates such as specified limits on data requirements or deadlines set in motion once an assessment is triggered. Collectively, these have created confusion and misunderstanding of what risk assessment is and how it ought to be performed. Parallel to this, science and science policy have advanced markedly. Precision in measuring chemical concentrations in various media has increased several-fold. Advances in molecular analysis (e.g., genetics, genomics, proteomics, metabolomics, high-throughput *in vitro* screening, eDNA and etc.) provide new insights into organismal biology and functional ecology. Computational capabilities have expanded by orders of magnitude and incorporation of New Approach Methods (NAMs), requires systematic consideration. Guidance has provided for additional assessment endpoints (e.g., ecosystem services) and has incorporated advances in understanding landscape interactions and metapopulation dynamics (see Canada's Federal Contaminated Sites guidance 2012). This presentation will detail advances in policy, guidelines, and science especially over the past dozen years and will explore persisting impediments for widespread adoption of recent advances in scientific understanding of how social-ecological systems operate.

03.05.06 Wildlife Toxicity Thresholds - EcoSSLs Revisited

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Ecological risk assessments are intended to assess the potential for a stressor (e.g., a metal) to present risk to an entity of interest (e.g., bird or mammal species). The screening level assessments that are frequently performed are based on hazard quotients (HQs) derived from a comparison of soil concentrations to EPA ecological soil screening levels (Eco-SSLs) for plants, invertebrates, birds, and/or mammals. These HQ values often become triggers either for remediation or further assessment. When HQs are derived using Eco-SSLs they are conservative by design and are intended to screen out contaminants that require no further evaluation. The Eco-SSLs for metals are low and often near background concentrations. This can result in very few metals being screened out, thus requiring that a baseline (retrospective) risk assessment is performed where an in-depth assessment of toxicity and exposure is performed. To assist with baseline risk assessments we reviewed the literature for toxicity studies on the following eight metals for plants, invertebrates, birds, and mammals: arsenic, cadmium, copper, lead, manganese, molybdenum, selenium, and zinc. Toxicity data for plants and invertebrates are based on soil or tissue concentrations, while toxicity data for birds and mammals are based on concentrations in their diets (or expressed as doses on a per mass of body weight basis). Toxicity reference values (TRVs) were developed from studies with concentration-response (or dose-response) data for endpoints related to survival, growth, or reproduction. In contrast to Eco-SSL TRVs, they are not derived using NOAELs (no observed adverse effect level) or LOAELs (lowest observed adverse effect level). TRVs are presented as EC_{10}, EC_{20} and EC_{50} (or ED_{10}, ED_{20}, ED_{50}) values.

03.05.07 Interspecies Scaling Approaches for Human and Ecological Risk Assessment of Organic Chemicals

P.C. Fuchsman, H. Clewell, Ramboll / Environment and Health

Risk assessments that focus on anthropogenic chemicals in environmental media—whether considering human health or ecological effects—often rely on toxicity data from experimentally studied species to estimate safe exposures for species that lack similar data. Current default extrapolation approaches used in both human health risk assessments (HHRAs) and ecological risk assessments (ERAs) account for differences in body weight between the test organisms and the species of interest, though the two default approaches differ in important ways. HHRAs currently employ a default based on body weight raised to the three-quarters power, whereas typical ERAs implicitly use body weight raised to the power of one. Interspecies extrapolation of wildlife TRVs typically has not been based on the same rigorous scientific consideration as has been undertaken to support HHRA and veterinary practice. As a result, ERAs tend to overestimate risks to small birds and mammals while potentially underestimating risks to large animals. Improved accuracy of interspecies extrapolation would result in sounder decision-making for contaminated sites, both by improving protection for large animals and by limiting ecological costs (e.g., physical habitat degradation, carbon footprint) and financial costs of excessive cleanup beyond what is truly needed to protect small animals. Toward that end, improved approaches can be implemented to take better advantage of available methods and data. Our review describes differences in the experimental data underlying various default practices and discusses the many factors that impact interspecies variability in chemical exposures; the interplay of these different factors can lead to substantial departures from default expectations. Alternative methodologies for conducting more accurate interspecies extrapolations in ERAs for wildlife are discussed, including: (1) physiologically based toxicokinetic (PBTK) modeling and (2) a system of categorical defaults based on route of exposure and toxic mode of action.

03.05.08 Tackling Uncertainty, Assumptions, and Unknowns in Current Wildlife Exposure Assessments

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A SETAC Technical Workshop on 'Wildlife Risk Assessment in the 21st Century: Integrating Advancements in Ecology, Toxicology, and Conservation" delivers a collective effort to provide guidance to advance current retrospective and prospective exposure assessment methods towards improved ecological risk assessment for wildlife in the 21st century. Exposure assessment paradigms and procedures have been developed during the last century in an attempt to minimize impacts on wildlife or human health caused by the use and misuse of chemicals, accidental events, and industrial activities. Although repeatedly improved, we highlight current methods for assessing exposure that remain constrained by the limitations of overly simplified models and methods, and incomplete data that are not well incorporated under current regulatory frameworks. Ultimately, ecological risk assessors are unable to fully achieve the goals of wildlife risk assessment and protection given the uncertainty, assumptions and unknowns in the current process. The use of hazard or risk quotients in wildlife screening assessments is based on implicit assumptions about the frequency, duration and intensity of wildlife exposure to single contaminants often using imperfect information on concentrations in the environment, major exposure routes, bioavailability and bioaccumulation for a "representative species/receptor". This leads to uncertainty that is seldom explicitly characterized or inadequately quantified, for example, using sensitivity analysis. There is a general lack of information on the occurrence, intensity, frequency and composition of mixtures, potential synergistic or antagonistic interactions with other stressors, and understanding of potential trophic interactions that can influence exposure beyond the representative species. This information is required to more efficiently link empirically derived individual-based exposure data to predict population and community responses. Regulators also need accessible decision support tools and models that better use the available lab and field data at both screening and higher tier assessments, while further reducing animal use. Our team is focused on addressing the identified limitations and providing concrete guidance for improving wildlife exposure assessment that promotes simplicity, is transparent about uncertainty, offers greater ecological relevance, and aligns with regulatory frameworks.

03.05.09 Guidance and Innovations for Improving Screening-Level and Refined Exposure Assessments for Wildlife

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A SETAC Technical Workshop on 'Wildlife Risk Assessment in the 21st Century: Integrating Advancements in Ecology, Toxicology, and Conservation" is developing recommendations to improve risk estimates by providing data, tools, and guidance to characterize chemical exposures to wildlife for both retrospective and prospective assessments. In this presentation, we offer guidance for improving wildlife exposure assessment for different applications (e.g., contaminated sites, pesticides) using tools and methods that fit within existing regulatory frameworks. The recommendations are intended to advance the scientific power of all tiers of the risk assessment process. There have been many scientific advances that enable improvements in the exposure portion of wildlife risk assessments. Examples for screening-level assessments include the development of simple dose and body burden-based models to address non-dietary routes of exposure (e.g., dermal exposure) and bioaccumulation. Possible improvements can be incorporated in refined exposure assessments including use of spatially- and temporally-explicit models and probabilistic techniques; accounting for bioavailability and bioaccessibility, and the interplay between different stressors; and use of physiologically-based pharmacokinetic data and models, and omic and trait-based approaches. Biomonitoring and other field-based tools including those that characterize exposure with fewer animals (e.g., passive samplers) have also substantially advanced to enable evaluation of exposure models and predictions. As the data, tools, guidance and ability to adapt improve, wildlife exposure assessment will move closer to the goals of accurately predicting upper bound exposure for screening-level assessments and a closer approximation of reality and variability of exposure in refined assessments.

03.05.10 Molecular and Pathway Approaches, Non-Standard Endpoints, and Statistical, Modeling and Read-Across Methods for 21st Century Wildlife Risk Assessment

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Despite advances in molecular toxicology, development of adverse outcome pathways, increased recognition of behavioral and physiological endpoints, and new statistical tools and modeling approaches, ecological risk assessment methods that evaluate effects of pesticides, metals, industrial chemicals, and other environmental contaminants on wildlife have remained largely unchanged for decades. While data on survival, growth, and reproduction in domesticated birds and rodents derived from historically used laboratory tests remain central to wildlife risk assessments, uncertainty may be reduced and clarified, and ecological relevance enhanced, by new approach methodologies, additional whole animal measurement endpoints, and improvements in the derivation of effect thresholds and their extrapolation to diverse species. Such improvements could enhance decision support tools within (often tiered) regulatory frameworks while maintaining simplicity. In this session, we present preliminary findings of the "Wildlife Effects Assessment" workgroup of the SETAC Wildlife Ecological Risk Assessment Workshop that address: (1) translation of new methods (e.g., cell/pathway-based approaches, toxicokinetics/toxicodynamics) to supplement or replace data derived from whole animal studies; (2) incorporation of other "non-standard endpoints"(e.g., behavior, genotoxicity, histopathology, endocrine disruption, immune modulation, metabolic disorders) derived from laboratory trials and field studies to support risk assessments and their ecological relevance; and (3) use of statistical methods (e.g., alternatives to null-hypothesis significance testing, improvements to toxicity reference value derivation), modeling (e.g., QSARs, species sensitivity distributions), and use of read-across methods that might improve estimation of risk among species, sites, and chemicals. Our preliminary findings and recommendations may enhance lines of evidence used in wildlife risk assessments such that uncertainty is reduced or better quantified, and ultimately the robustness of regulatory decisions to achieve wildlife protection goals is improved. The views expressed in this abstract 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.

03.05.11 Protecting Terrestrial Wildlife in the 21st Century: Are Current Wildlife Toxicity Testing Protocols and Animal Models the BEST We Can DO?

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Model species such as the northern bobwhite and mallard, as well as laboratory species such as domesticated rats and mice, have been employed in regulatory tests for decades to generate data used in risk assessments for terrestrial mammals, birds, and even reptiles and amphibians. While it is important to be able to conduct the hazard component of wildlife risk assessments using species that can tolerate laboratory conditions, and for which supporting data are available, there are many knowledge gaps when extrapolating data from laboratory models to the diverse range of terrestrial wildlife that may be considered in these risk assessments. This has led to a regulatory framework where data collected in model species are used as surrogates for all terrestrial vertebrates, using uncertainty factors to account for any intra- and inter-species differences. There is an assumption that the effects detected in the model species in the laboratory are accurate, relevant, and reproducible across species and scenarios; i.e., effects on survival, growth, and reproduction in a model species in the laboratory translate to population-relevant effects in free-ranging species. While uncertainty factors add a degree of conservatism to this framework, it remains challenging to verify and ground truth such factors. While field data and modelling approaches offer the opportunity to refine risk assessments at higher tiers (e.g., examining exposure and effects in actual species likely to be present at a contaminated site), the protocols for such investigations are not standardized. Guidance on how to conduct and interpret results from tests that could be used in field settings remains a significant information gap; conversely, field data can also be used to redefine compound-specific laboratory tests. Here we present preliminary findings from activities of the "Wildlife Effects Assessment" workgroup of the SETAC Wildlife Ecological Risk Assessment Workshop to address: (1) availability and suitability of existing standard wildlife toxicology testing protocols to support risk assessments and decisions, and (2) animal models and interspecific data extrapolation for wildlife risk assessments. The workgroup aims to provide recommendations that could be used to create a more ecologically relevant risk assessment framework with greater quantification of uncertainties, incorporating approaches that use fewer animals than at present and while retaining an element of simplicity. The views expressed in this abstract 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

03.05.12 Monitroing Indicators of Chemicals Exposure and Effects in the Terrestrial Environment, in England

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This paper is submitted in memory of the late Prof Richard Shore. As part of its 25-year Environment Plan (25-YEP) the UK government aims to reduce chemical exposure and its effects on wildlife. The indicators used to monitor progress towards the goal of 'managing exposure to chemicals' will cover 3 environmental components: terrestrial, aquatic and marine. Progress on the development of the indicator's terrestrial component will be discussed, showing our 'interim' version, which uses a dashboard approach. The interim terrestrial indicators are based on chemical concentrations found in the environment and in different organisms including sparrowhawk (*Accipter nisus*), red kite (*Milvus milvus*), and red foxes (*Vulpes vulpes*). The chemicals presented are representative of 3 groups requiring priority management as highlighted in the 25-YEP: persistent, bioaccumulative and toxic (PBT) substances; heavy metals; and pesticides/biocides. The dashboard illustrates significant trends over time in environmental concentrations for the presented chemical/receptor combinations. The indicators also consider potential risks to wildlife from chemicals by comparing the most-recent year(s) concentration data against relevant environmental protection thresholds, where available. This assessment of risk provides a surrogate for effects reporting for this interim indicator. Development of the indicator suite is ongoing to improve our ability to report trends in exposure of wildlife, at multiple trophic levels, for prioritised substances (incl. a 4th group of 'chemicals of emerging concern'), and to consider possible indicators that could be included to reflect direct and indirect, as well as lethal and sublethal effect endpoints.

03.05.13 Terrestrial Monitoring of Authorised PPPs and Their Effects

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This paper is submitted in memory of the late Prof Richard Shore. In the UK, the authorisation of plant protection products (PPPs) involves conducting prospective environmental risk assessment involving a comprehensive suite of ecotoxicology, fate, and behaviour tests and, particular higher tier risk assessment, field studies aimed at refining the risk to key species. Post registration residues of pesticides (PPPs and other compounds) are monitored in surface (and ground) waters to monitor compliance with the European Union Drinking Water Directive, but also via wildlife incident schemes (e.g. Fish kills). However, there is no equivalent residue monitoring for PPPs in the terrestrial environment, reflecting the lack of a legislative requirement for such measurements. There are however, both "incident based" investigative monitoring (e.g. Wildlife Incident Investigation Scheme (WIIS) looking at mortality incidents in pollinators or wild vertebrates), and "opportunistic based" monitoring schemes (e.g. Predatory Bird Monitoring Scheme (PBMS)). These are the only UK schemes that attempt to assess the exposure and possible direct effect of PPPs on terrestrial wildlife. The findings of a review undertaken by UK CEH will be published in a Natural England Report. It will be used to develop proposals for consideration, by the UK Department for Environment, Food and Rural Affairs (Defra) and the Health and Safety Executive's (HSE) Chemical Regulation Division, for wider post authorisation terrestrial monitoring of PPPs and their effects in wildlife. The proposed monitoring scheme in isolation will in most cases not provide diagnostic answers to whether PPPs are having an impact at the population, community or ecosystem level. Instead the ambition was that the programs within the scheme should be able to identify changes in the status of our terrestrial environment, or particular parts of it, at an earlier level of organization with a potential to provide early warnings. With such observed changes in status of components being viewed as a trigger for further monitoring, investigation and potential review. A number of schemes that could contribute to the proposed monitoring scheme are discussed.

03.05.14 MicroRNAs as Biomarkers of Immunomodulation in Migration Champions

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Pollutant exposure has been linked to immunosuppression and disease outbreaks in various wildlife species. Yet, our understanding of the molecular mechanisms involved in pollutant-induced immunomodulation remains limited. Recently, microRNAs have been identified as key regulators of the immune response and important determinants of disease outcomes. MicroRNAs are a class of small noncoding RNAs that bind specific mRNA transcripts (from the host cell as well as from viruses) and prevent their translation into proteins. Alterations in microRNA expression have been found after exposure to various pollutants and pathogens (e.g. influenza virus). Recent in vitro studies have demonstrated that pollutants deregulate microRNA pathways involved in antiviral responses. MicroRNAs might therefore be a mechanistic link in our understanding of pollutant-induced immunomodulation. The presence and stability of microRNAs in blood makes them potentially interesting biomarkers of pollution-induced disease outcomes. As part of the COAST IMPACT project, we study the potential of microRNAs as biomarkers of pollution exposure and disease in migratory shorebirds of the East Asian Australian flyway (EAAF). EAAF shorebirds are facing significant conservation threats and many populations are declining. The role of pollutant exposure in these declines remains unclear. The detrimental effects of pollution exposure might further be exacerbated in these birds due to their energy-demanding annual extreme migrations, where lipophilic pollutants can redistribute in tissues and potentially cause toxicity. Next-generation sequencing was used to characterise the microRNA profiles in serum from shorebirds with different avian influenza virus status. We will present potential target sequences of the identified serum microRNAs in the shorebird transcriptome and virome. Furthermore, blood pollutant concentrations will be analysed alongside microRNA profiles to investigate potential microRNA signatures of pollution exposure. The identification of specific microRNA profiles associated with infectious disease and/or pollution exposure could (i) elucidate the molecular mechanisms inducing pollutant-induced immunomodulation, and (ii) facilitate the assessment of pollution exposure effects in free-living birds of conservation concern.

03.06 -Wildlife Ecotoxicology: Conservation and Regulatory Connections

03.06.02 Migration and Energetics Model Predicts Delayed Migration and Likely Starvation in Oiled Waterbirds

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Oil spills are known for inflicting mortality and injury on bird populations. Many of these deaths can be traced back to starvation resulting from thermoregulatory costs incurred by oiling of birds' feathers. However, the fates and responses of sublethally oiled birds are poorly known. Using mallard-like ducks as a model organism, we combined values from previous laboratory studies of the thermoregulatory costs of oiling with a modified version of an existing temperature-influenced avian migration energetics model that predicts energetics and migration patterns of an "average" bird. Using this model, we examined the potential effects of oiling on general migration patterns, changes in energetic gains necessary to compensate for oiling, and starvation. We assessed all metrics across multiple degrees of oiling and assessed starvation across both degrees of oiling and body condition. Median estimates for delays in spring migration were one to two months for trace and lightly oiled birds, respectively, and we predicted arrested spring migration in moderately oiled birds. Median estimates of required increases in energetic gains ranged from 20.3% to 88.6% depending on intensity of oiling. We predicted a high risk of starvation within four weeks for most combinations of oiling intensity and body condition at the median predicted wintering temperature of unoiled birds (-4.9°C). However, at the average temperature of the southernmost wintering latitude in winter (10.8°C), we predicted only moderately oiled birds in poor or good body condition were at risk of starvation within a four-week time frame. Due to the potential for even trace oiling to cause delayed spring migration and decreased body condition, sublethal oiling during spring migration could reduce a bird's reproductive capacity through thermoregulatory costs alone. Future research incorporating the components of this model into a population scale, spatially explicit migration model could provide additional insight into the potential effects of sublethal oiling on reproduction and survival and allow managers to more accurately assess both potential risk and resulting injury to bird populations impaired by sublethal oiling.

03.06.03 The Impact of Pesticide Toxicity on Bird Populations in England

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Farmland birds have declined considerably in England since the 1970s, generalist species have also experienced a decline but not as sharp. Agricultural intensification has been linked as the main driver behind the decline. The introduction of pesticides and fertilisers is one of the main contributors negatively impacting several farmland species. There is extensive research focusing on their effects on invertebrates such as pollinators but less is known about organisms at higher trophic levels. Previous modelling studies using long-term bird abundance data have found mixed results between the dietary exposure to neonicotinoids and farmland bird population changes. Studies like these are limited to a single species or pesticide class. It is important to consider the wider range of pesticides utilised on arable farmland to better understand the driver behind the immense decline in farmland bird numbers. Using bird abundance data and pesticide usage data of arable land for England at a 1x1km resolution, generalised linear mixed models were used to test for spatio-temporal associations between pesticide usage and changes in populations of 30 species (farmland and generalist) between 2007 and 2016. Model estimates were not consistent across all species, so we cannot conclude firmly if pesticides are the driving cause for bird declines however seven of eighteen farmland bird species present negative associations with at least one of 16 pesticides (goldfinch *Carduelis carduelis*, house sparrow *Passer domesticus*, jackdaw *Corvus monedula*, kestrel *Malagasy kestrel*, linnet *Linaria cannabina*, lapwing *Vanellus vanellus*, starling *Sturnus vulfaris* and woodpigeon *Columba palumbus*). Cyprodinil, prothioconazole and glyphosate potentially pose greater risk to bird species than other pesticides and, as expected farmland birds are at greater risk than generalist bird species – although other songbirds are still impacted.

03.06.04 Anticoagulant Rodenticides: Spatial and Temporal Trends in Terrestrial Birds of Prey From Western Canada

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As the dominant means for control of pest rodent populations globally, anticoagulant rodenticides, particularly the second generation compounds (SGARs), have widely contaminated non-target organisms. Here we present data on hepatic residues of anticoagulants in 746 raptorial birds found dead or brought into rehabilitation centers in British Columbia, Canada over a 30-year period from 1988 to 2018. The incidence and extent of exposure varied by species, geographic area, and over time, with at least one SGAR residue detected in 74% of all raptor livers sampled. Hepatic SGAR concentrations varied from < 0.005 to 1.83 mg/g wet wt. By comparison, first generation compounds (FGARs), typified by warfarin, were detected in < 5% of the raptors analyzed. Highest rates of exposure were in larger owl species with diverse diets, that can include targeted rats, and that have inhabited suburban and intensive agricultural habitats, particularly barred (*Strix varia*) and great horned (*Bubo virginianus*) owls. Overall exposure to at least one AR in those two species in the south coast Fraser Valley and Delta regions was 97% and 89% respectively. Barn owls (*Tyto alba*), mainly a vole (*microtus*) eater, had a lower incidence of exposure of 66%. Concentrations of SGARs were very variable, for example in Fraser Valley barred owls, the geometric mean = 0.14, with a range < 0.005 to 1.81 mg/g wet wt (N = 195). Putatively bird-eating raptors also had relatively high incidence of exposure with Cooper's hawks (*Accipiter cooperii*) at 75% and sharp shinned hawks (*Accipiter striatus*) 60%, peregrine falcons (*Falco peregrinus*), 75%. Trends in the incidence of exposure to summed SGARs or to any individual compound did not change significantly over the time period in either all raptors collectively or individual owls with adequate sample sizes. However, mean concentrations of bromodialone increased while brodifacoum and defethialone decreased coincident with implementation of regulatory restrictions on their usage commencing in 2012, which followed a trend among commercial applicators to move from brodifacoum to bromodialone about 2008.

03.06.05 Pharmaceuticals in the Environment: Exposure, Effects and Risk to Wildlife

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Pharmaceuticals and their metabolites are biologically potent compounds that have gained public attention in the past two decades due to widespread detection in the environment, and in a few instances, population level effects on fish and wildlife. Active pharmaceutical ingredients (APIs) are designed to affect specific biochemical, cellular, physiological, or behavioral processes. Many of the target receptors of APIs are evolutionarily conserved across species. In the United States alone, the FDA has approved over 17,000 different prescription drug products for human use and 1,600 animal drug products which include one or more of the approximately 4000 different APIs in use. There are numerous sources and convoluted exposure pathways by which wildlife could be exposed to APIs of human and veterinary origin: wastewater treatment plants (WWTP), landfills, farmland and forests irrigated with WWTP effluent, fields amended with biosolids, surface waters that receive effluents or runoff, and carcasses of API-treated livestock or companion animals. While many of these exposure pathways remain hypothetical, the most notable example of a population level effect on wildlife from any chemical was the dramatic collapse of Asian vulture populations (1990s to mid-2000s; >99% decline) due to veterinary use of the non-steroidal anti-inflammatory drug (NSAID) diclofenac. The story of the Asian vulture population collapse should serve as a warning to encourage us to improve our understanding of uncertainty in ecological risk assessment. Even if risk to wildlife from pharmaceuticals was assessed similarly to pesticides, the differential NSAID sensitivity among avian species combined with the unique societal and cultural circumstances in India related to the vultures would have made predicting the population level outcome using a prospective approach to risk assessment unlikely. Since then, regulatory and policy responses have been undertaken in Asia, a safe alternative (meloxicam) has been identified, and NSAIDs (including diclofenac, flunixin, carprofen, ibuprofen, phenylbutazone and ketoprofen) have been investigated in other species and geographic areas. Despite the collapse of Asian vulture populations, pharmaceutical exposure, hazard, and risk assessment for wildlife are not typically conducted by regulators. This presentation will review our current understanding of wildlife and pharmaceuticals in the environment, and identify future priorities for monitoring exposure, characterizing effects, and assessing risk.

03.06.06 Selenium and its Complex Relationship with Birds

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In birds, Selenum can induce non-reproductive chronic effects, impaired productivity through decreased fertility, and reduced egg hatchability and fatal embryo deformity. Here we examine the impacts on the American Dipper (Cinclus mexicanus) in fast-flowing mountain streams within the Elk Valley, BC, where the coal mining industry poses a variety of long-lasting impacts, including Selenium contamination, to surrounding aquatic ecosystems. The Elk Valley in southeastern British Columbia is currently host to five active open-pit coal mines and there are plans for substantial expansion. To minimize adverse effects in aquatic organisms, the British Columbia Ministry of Environment has set a selenium concentration guideline in the water column at 2μg/L. In 2016, water directly downstream of active mines, in their standard operation, shows high levels of selenium up to 39.8μg/L. However, the water concentration guideline is inadequate to quantify risk to the ecosystem, as it is greatly complicated by wide variation in biological uptake and selenium speciation. We describe the reproductive status and contaminant levels in American Dippers in this system in the context of risk assessments for these birds and other species here and elsewhere.

03.06.07 How Regulations Need to Change to Protect Science and Wildlife and Human Health: A Case Study on the Bent Science Surrounding Atrazine

J.R. Rohr, University of Notre Dame / Department of Biological Sciences

In toxicology, practitioners and scientists spend careers deliberating and even debating safety factors, how to conduct ecological risk assessments, and relevant exposure concentrations of toxicants to humans and wildlife. While all of this is important, rarely do toxicologists and regulators discuss an even more important, but not uncommon, phenomenon in toxicology, 'bending science', defined as manipulating research to advance economic, political, or ideological ends. Bending science includes tactics such as creating misleading science, suppressing and attacking undesired science, harassing scientists, manufacturing uncertainty, and packaging and spinning science. Bending science adversely influences the quality and type of data that make it through the pipeline to regulators, as well as the pipeline of scientists willing to enter or stay in toxicology. In this talk, I will provide several examples of either bent science or ethically questionable decision making made by members of industry, government, the legal system, and institutions of higher education in the history of research and regulations surrounding the herbicide atrazine. Importantly, these decisions likely had consequences for wildlife and human health and in many cases served to erode public trust in the discipline of toxicology, science in general, and the honorable functioning of societies. Most importantly, I will offer several recommendations that should help to reduce bent science, enhance the credibility and integrity of toxicology and science more generally, and enrich human and environmental health.

03.06.08 The Lead (Pb) Lining of Agriculture-Related Subsidies: Enhanced Golden Eagle Growth Rates Tempered by Pb Exposure

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Supplementary food resources (e.g., subsidies) associated with agriculture can benefit wildlife species, increasing predictability and availability of food. Avian scavengers including raptors often take advantage of subsidies associated with both recreational hunting and pest shooting on agricultural lands. However, these subsidies can contain lead (Pb) fragments if they are shot with Pb-based ammunition, potentially leading to Pb poisoning and physiological impairment in wildlife. Nesting Golden Eagles *(Aquila chrysaetos*) commonly forage in agricultural lands during the breeding season, and therefore, both adults and their nestlings are susceptible to Pb exposure from scavenging shot wildlife. We assessed Pb exposure in 258 nestling Golden Eagles (401 total blood samples), along with physiological and growth responses, in agricultural lands across four western states in the United States. We also evaluated the birds' Pb stable isotope signatures to inform exposure sources. Twenty-six percent of Golden Eagle nestlings contained Pb concentrations associated with subclinical poisoning for sensitive species (0.03–0.2 µg/g ww), 4% exceeded subclinical poisoning benchmarks (0.2–0.5 µg/g ww), and < 1% exceeded concentrations associated with clinical poisoning (0.5–1.0 µg/g ww) or those considered to cause severe clinical poisoning (>1.0 µg/g ww). Lead concentrations were highest in nestlings with close proximity to fields that potentially provided subsidies and declined exponentially as distance to subsidies increased. However, close proximity to agriculture, and presumably subsidies, positively influenced nestling growth rates. Across the range of Pb exposure, nestlings experienced a 67% reduction in delta-aminolevulinic acid dehydratase activity, suggesting exposed nestlings may have been anemic or experiencing cellular damage. Isotopic ratios of 206Pb/207Pb increased non-linearly with increasing blood Pb in Golden Eagle nestlings, and 45% of the birds were consistent with those of ammunition. However, above 0.10 µg/g ww, the proportion associated with ammunition increased to 89% of the nestlings. An improved understanding of how these positive (growth) and negative (physiology) effects associated with proximity to subsidies interact would be beneficial to managers when evaluating potential measures to reduce Pb exposure across the landscape.

03.06.09 Tracing the Sources of Tree and Barn Swallow Neonicotinoid Exposure in Prairie Agroecosystems

M. Harris, University of Saskatchewan / Department of Biology; K.A. Hobson, University of Western Ontario / Department of Biology; C.A. Morrissey, University of Saskatchewan / Department of Biology

Populations of farmland-breeding aerially insectivorous birds have declined significantly across Canada, but the causes of these losses are unclear. Though chronic dietary insecticide exposure has been proposed as a driver of declines, pesticide exposure in this guild is largely unstudied. This project will quantify the neonicotinoid insecticide exposure of two sympatric declining aerial insectivores, the Barn Swallow (*Hirundo rustica*) and the Tree Swallow (*Tachycineta bicolor*), breeding across a gradient of agricultural intensity in Saskatchewan, Canada. We are examining whether differences in foraging location or diet influence neonicotinoid exposure, and hypothesize that use and availability of aquatic and terrestrial resources will result in different patterns of exposure across the land use gradient. In 2020 and 2021, we collected foraging data from adult swallows for one day during peak chick rearing using Pinpoint GPS tags. We collected blood samples from adults and nestlings to assess neonicotinoid exposure, as well as fecal samples to evaluate diet composition through DNA metabarcoding. Preliminary results indicate that foraging behavior differed between species, with Tree Swallows selecting for wetlands more strongly than Barn Swallows. Despite Tree Swallows' greater use of wetlands, both species exploited emergent aquatic insects, with nestlings in particular disproportionately consuming emergent insects in the families Tipulidae (appearing in 26% of nestling and 0% of adult samples) and Limoniidae (35% of nestling and 15% of adult samples). Although analysis of blood neonicotinoid exposure is still pending, these preliminary foraging and diet findings will improve our ability to identify sources of insecticides. By increasing our understanding of the relative contaminant contributions of terrestrial versus aquatic inputs, we can better characterize exposure risk within the rapidly declining aerial insectivore guild.

03.06.10 A Battle for Burden of Proof: Neonicotinoid Science and Regulation in Canada

C.A. Morrissey, University of Saskatchewan / Department of Biology

On March 31 and May 19, 2021, Health Canada's Pest Management Regulatory Agency (PMRA) released their final decision on the re-evaluation of neonicotinoids clothianidin, thiamethoxam and imidacloprid where they approved the continued registration with some minor use cancellations for select vegetables and fruits and crop specific mitigation measures (e.g. toxic labelling requirements, rate reductions, spray buffer zones). This reversed PMRA's earlier 2016 proposed decision to phase out all outdoor uses of neonicotinoid products due to "unacceptable risk to aquatic organisms" and followed a shift from a separate 2019 Pollinator Assessment that deemed risks were now acceptable. This most recent controversial decision, which was an apparent flip-flop, represents a familiar scenario for pesticide registration in North America. Here I highlight how this protracted regulatory process for neonicotinoids has led to an increased burden of proof on scientists battling a familiar industry-led effort to bend the science. After delaying the decision, large amounts of new data rapidly accumulated on the toxicity to aquatic organisms and seed eating birds as well as industry led water monitoring across the vast Canadian agricultural landscape which was made available to regulators. However, surface water detections and exceedance of aquatic toxicity thresholds appear to show bias of the data provider (industry vs non-industry) and there was inconsistent interpretation of the data by crop. Further challenges in determining established toxicity thresholds for aquatic ecosystems and birds using HC5 values from established Species Sensitivity Distributions also resulted in changes to data inclusion thereby reverting to older methods based on the most sensitive species in some cases with only 6-7 aquatic invertebrates and 3 bird species. A screening level assessment of imidacloprid for avian wildlife indicated all crops and uses were exceeding the level of concern with risk quotients >>1 based on consumption of treated seeds that typically represent < 1-2% of the diet. However, claims about treated seed availability, lack of mortality incidents, field dissipation, and "learned avoidance studies" were used to support a risk characterization that contradicts the science on real world avian exposures. This presentation represents a call to action to increase the speed of regulatory decisions for pesticides, remove industry influence on the regulatory process, and standardize wildlife risk assessment practices based on the best available peer reviewed science to improve decision making and transparency.

03.06.11 Science in Support of Recovering At-Risk Cetaceans

T.M. Brown, Fisheries and Oceans Canada / Government of Canada / Ocean Sciences Division

The *Endangered* transboundary Southern Resident Killer Whales (SRKW, *Orcinus orca*) and St Lawrence Estuary Beluga Whales (SLE beluga, *Delphinapterus leucas*) face significant threats from high levels of endocrine disrupting contaminants, alongside other conservation threats including noise, disturbance, vessel strikes and decline in prey abundance. Prioritizing those contaminants that present health risks to high trophic level marine mammals is exceedingly difficult, especially those that are classified as endangered. Under the Canadian *Whales Initiative*, Fisheries and Oceans Canada (DFO) and other partners are leading efforts to reduce the release of contaminants of concern, as well the other anthropogenic threats to these populations. Since January 2019, DFO has been conducting research to inform actions to recover the SRKW and SLE beluga. Research is focused on the whale populations and their prey in designated *Critical Habitat* and surrounding habitats in partnership with First Nations, NGOs, universities and other agencies. In the case of SRKW, an extensive study of contaminants and their effects on the whales, Coho salmon (*Oncorhynchus kisutch*) and their primary prey, Chinook Salmon (*Oncorhynchus tshawytscha*) is underway, entailing research in both freshwater and marine habitats for the salmonids. In the case of SLE beluga whales, efforts are focused on studying contaminants and their effects on the whales and identifying contaminants in their habitat (i.e. sediments) and putative (and poorly understood) prey, including Atlantic cod (*Gadus morhua*), Greenland cod (*Gadus ogac*), redfish (*Sebastes* sp.), capelin (*Mallotus villosus*), polychaetes and shrimp. The ultimate goal is to establish a risk-based list of priority contaminants that threaten the whales and/or their prey, and to act on the outcomes in support of a wider conservation agenda for these charismatic species.

03.07 Wildlife Ecotoxicology: Exposure and Effects of Chemical Pollutants on Wildlife in Laboratories and Natural Environments

03.07.02 Plastic Additive Impacts on Marine Mammal Physiology

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As microplastic pollution continues to increase, organisms are increasingly being exposed to additives used in their manufacturing, such as the plasticizers bisphenol-a (BPA) and bisphenol-s (BPS), which are released into the environment as plastics degrade. Studies in humans and laboratory rodents have shown that BPA and BPS are endocrine disruptors associated with reproductive and neural abnormalities. These additives bioaccumulate in marine ecosystems and can have potentially magnified adverse effects on marine mammals, which are already exposed to multiple other anthropogenic stressors (e.g. disturbance, climate change). However, the biological effects of BPA and BPS on marine mammals, whether alone or in combination with other stressors, have not been studied. To address the practical and ethical challenges of directly assessing the impacts of contaminants on large marine mammals, we used an ex vivo approach to examine the combinatorial impacts of stress hormones and plasticizers on marine mammal tissue. We prepared precision-cut explants of blubber tissue, the primary energy depot and reservoir of lipophilic pollutants in marine mammals, collected from free-ranging northern elephant seals (NES; n = 3). We exposed blubber explants to cortisol, epinephrine, and BPA and BPS alone and in combination in culture, and assessed their effects on the blubber transcriptome. We found that the transcriptional response of blubber explants to cortisol recapitulated that observed in whole-animal experiments, validating the utility of the ex vivo approach for functional studies in marine mammals. While exposure to BPA and BPS alone had negligible effects on the blubber transcriptome, the plasticizers significantly impacted tissue responses to epinephrine by altering expression of genes associated with lipid homeostasis, adipogenesis, nuclear receptor signaling, and inflammation. Together, our data suggest that plasticizers, which may be stored in and subsequently released from blubber tissue during stress-induced lipolysis, may alter tissue and energy homeostasis and impact the ability of marine mammals to respond appropriately to natural and anthropogenic stressors.

03.07.03 Associations Between Anthropogenic Chemical Pollutants and Hormones in Primates Inhabiting Kibale National Park, Uganda

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Despite the thousands of anthropogenic chemicals on the market today and an increasing awareness of the hazards associated with their release into the environment, our understanding of physiological effects of exposure to chemical pollutants in wildlife is limited. Wildlife biomonitoring provides an alternative to laboratory-based toxicity tests, integrating ecological factors and real-world complexities unattainable in controlled settings. In this study, we use noninvasive biomonitoring to examine relationships between fecal concentrations of 100+ chemical pollutants, including organochlorine pesticides (OCPs), brominated flame retardants (BFRs) and organophosphate esters (OPEs), and fecal hormone metabolites of the hypothalamic–pituitary–adrenal (HPA) (*i.e.,* cortisol), hypothalamic–pituitary–gonadal (HPG)(*i.e.,* testosterone, estradiol), and hypothalamic–pituitary–thyroid (HPT) (*i.e.,* triiodothyronine) axes in 76 samples across four species of wild nonhuman primates inhabiting Kibale National Park in western Uganda, including chimpanzees (*Pan troglodytes schweinfurthii*), olive baboons (*Papio anubis*), red colobus (*Piliocolobus tephrosceles*), and red-tailed monkeys (*Cercopithecus ascanius*). Our results show that concentrations of OPEs are the most abundant across primate species, with the highest concentrations found amongst red colobus species and in the adult male age/sex category. We found positive associations between OCPs ($R2 = .443$, $p = 0.021$), BFRs ($R2 = 0.323$, $p = 0.0040$), and OPEs ($R2 = .309$, $p = 0.055$) and fecal cortisol across all juveniles, as well as positive associations between OCPs ($R2 = 0.323$, $p = 0.0040$)and OPEs ($R2 = .508$, $p = 0.0104$) and fecal estradiol across all juveniles. Given the critical roles of the HPA and HPG axes on fitness, an understanding of exogenous chemical stressors on these networks can aid in conservation efforts of wildlife populations, while also providing insight into health threats for other species, including humans.

03.07.04 Tissue Distribution of Short- and Long-Chain Perfluoroalkyl Acids in Tree Swallows and Insights Into Dietary Uptake Using Fatty Acid Signatures

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Perfluoroalkyl acids (PFAAs) are highly stable terminal acids, which along with their per-/poly-fluoroalkyl substance (PFAS) precursors, are produced in large quantities and are widely used in industrial and commercial applications. Short-chain PFASs are defined as < 6 carbons for perfluorosulfonic acids (PFSAs) and < 7 for perfluorocarboxylic acids (PFCAs). With the restriction and phase out of long-chain PFAAs (> 7 carbons), such as perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) and their related chemistries, under the UN Stockholm Convention, production and use of PFAAs has shifted towards the short-chain homologues. Unlike long-chain PFAAs, the environmental behavior of short-chain PFAAs is relatively less known, including their tissue distribution in biota and associations with feeding habits. The objectives of this study were to compare the distribution of a suite of (potentially) bioaccumulative short-chain and long-chain PFAAs among tissues of wild tree swallows (*Tachycineta bicolor*) and to assess correlations between hepatic PFAA concentrations and hepatic fatty acid signatures, which are chemical tracers of dietary consumption patterns. The profiles and distribution of 16 PFAA homologues, including short-chain PFAAs (PFSAs, e.g., PFBS, PFHxS) and long-chain

PFAAs (PFCAs, e.g., PFHpA, PFOA, PFNA, PFDA, PFUdA, PFDoA, PFTrDA, PFTeDA, PFHxDA) were examined in tree swallow eggs and nestlings (liver, whole carcass) in southern Ontario, Canada. Freshly laid eggs (N = 20) and two 10-day-old sibling chicks (N = 40) were collected from 20 nests. In general, eggs, which reflect maternal burdens, had significantly higher concentrations of sum (∑) PFAAs than nestling livers and carcasses. ∑PFSA concentrations were significantly higher than ∑PFCAs in eggs and livers but similar in carcasses. PFOS, PFDS, PFNA, PFDA, PFUdA, PFDoA, PFTrDA, PFTeDA, and PFODA had significantly higher concentrations in eggs compared to nestling tissues, whereas PFBS, PFPeA, PFHpA, and PFOA had significantly higher concentrations in nestling tissues than eggs. The predominant PFAAs varied somewhat among the three types of tissues: in descending order, the egg profile consisted of PFOS>PFDA>PFUdA>PFNA, the liver profile of PFOS>PFNA>PFDA>PFOA, and the carcass profile of PFOS>PFNA>PFOA=PFDA. Hepatic ∑PFSAs and ∑PFCAs in tree swallows was significantly associated with diet, as determined by hepatic fatty acids.

03.07.05 Kinetics and Toxicity of PFAS in Peromyscus

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Ecological risk concerns about per- and polyfluoroalkyl substances (PFAS) stem from their ubiquitous presence in the environment, persistence, and variable/uncertain bioaccumulation and toxicity. In the present work, five perfluoroalkyl acids (PFAAs) and one fluorotelomer were administered to white-footed mice (*Peromyscus leucopus)* to elucidate if plateaus in serum concentrations were reached following 28 days — an exposure period common in toxicity testing. Blood samples from 14 or 21, and 28 days of exposure were analyzed for individual PFAS concentrations via liquid chromatography-tandem mass spectrometry (LC-MS/MS). Reproductive, developmental, and immunological endpoints were evaluated in *Peromyscus* exposed to PFOS, PFNA, PFHxS, and 6:2 FTS. In general, a plateau in serum PFAS concentration of *Peromyscus* orally exposed to PFAS for 28-days depends on interactions among (i) PFAS identity, (ii) continuous vs. bolus dosing, and, to a lesser extent, (iii) sex. Based on median values, the serum-to-dose (ng-PFAS/mL-serum / kg-PFAS/mg-animal) ratios from lowest to highest are: 6:2 FTS, PFBS, PFOA, PFHxS, PFNA, and PFOS. As such, greater levels of PFOS relative to amount of oral dose delivered were observed in serum than any other PFAS tested. In the reproductive and developmental tests, we observed effects of PFOS, PFNA, PFHxS, and 6:2 FTS on population-relevant endpoints such as neonatal survival and immune response. Then, serum levels from our toxicokinetic studies were used to estimate internal dosimetry-based wildlife Toxicity Reference Values (TRVs). To our knowledge, these are the first comprehensive short and long-term toxicity/toxicokinetic tests for any PFAS with a North American native mammalian species. Given the importance of internal dosimetry in accurate extrapolation across species, the present work is valuable for improving the accuracy of ecological risk assessment.

03.07.06 Parent and Alkylated Polycyclic Aromatic Compound Residue in Avian Tissue Following Diluted Bitumen Exposure

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Canadian petroleum production and exports are predominantly unconventional crude products from the country's oil sands region. Pipeline capacity expansions will likely increase the amount of one such ubiquitous oil sands crude product called diluted bitumen (dilbit) that is transported throughout the Pacific Northwest. In the event of dilbit spills, avian petroleum exposure estimates available in the literature are scare but indicate that ingestion of up to several ml oil / kg body weight (bw) / d could occur in highly exposed adult birds and at least tenths of mg oil / g onto the eggs of breeding birds should they become oiled and contaminate their eggs. In this context, we present tissue residue data from captive dosing studies with 2–12 ml dilbit / kg bw / d in adult zebra finch (*Taeniopygia guttata*) and 0.1–0.4 mg dilbit / g egg with double-crested cormorant (*Phalacrocorax auritus*) embryos. Parent and alkylated polycyclic aromatic compound (PAC) profiles in those matrices show qualitatively elevated PAC concentrations in only the most highly exposed dose groups. This suggests that birds in our studies were efficiently biotransforming and eliminating many of the analyzed PACs in dilbit under all but the most severe exposure scenarios. This study is the first tissue PAC residue data available for avian exposure to a bulk oil sands bitumen product.

03.07.07 Exposure to Halogenated Flame Retardants in Urban-Adapted Gulls: Is the Atmospheric Exposure Pathway Underestimated?

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Elevated levels of polybrominated diphenyl ethers (PBDEs) and selected emerging halogenated flame retardants (HFRs) have been reported in ring-billed gulls (Larus delawarensis) nesting in the Montreal area (QC, Canada). These urban-adapted birds forage in local waste management facilities such as landfills, where elevated air HFR concentrations can be found. Gulls can thus be exposed to HFRs through multiple pathways including diet, inhalation (gas- and particle-phase), and ingestion during preening of HFR-laden particles that are sorbed onto their feathers. However, the contribution of these different exposure pathways in birds remain largely unknown. Our study aimed to investigate the contribution of different exposure pathways to PBDEs and selected emerging HFRs in ring-billed gulls breeding in the Montreal area. Gulls were equipped with a miniature passive air sampler (PAS) fixed on their back along with a GPS datalogger for ten days. PBDEs and emerging HFRs were analyzed in PASs and in plasma, lung, feather surface, preen oil, liver, and stomach content of 26 ring-billed gulls. Results showed that BDE-209, -47 and -99 were predominant in PASs and all tissues, except for preen oil for which BDE-209 was less abundant than BDE-47 and -99. The levels of BDE-209, -47 and -99 measured in PASs were positively correlated with their concentrations in lungs and on the surface of feathers. They were also associated with those in liver, suggesting that atmospheric exposure of PBDEs may result in accumulation in liver. However, no relationship was found for BDE-209 concentrations between stomach content and liver, while BDE-47 and -99 concentrations in these two tissues were positively correlated. Therefore, unlike BDE-47 and -99, the exposure pathway of BDE-209 through inhalation and indirect ingestion of particles adhering on bird feathers were more important than direct dietary ingestion. This study demonstrated that ring-billed gulls in the Montreal area are exposed to PBDEs via both dietary and atmospheric (i.e., mainly BDE-209) exposure. We conclude that atmospheric exposure should not be underestimated compared to the traditional dietary route of exposure for urban-adapted birds.

03.07.08 Polychlorinated Biphenyl (PCB) Exposure Assessment of Carolina Wren and Barn Swallow Eggs and Nestlings Collected Along Choccolocco Creek Watershed, Alabama

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Decades after the halt in production of polychlorinated biphenyls (PCBs), wildlife connected to aquatic food webs near a production facility remain exposed to these chemicals and associated substances. The persistence of PCBs in aquatic ecosystems and exposure pathways for wildlife are well established. Our study was designed to quantify PCB exposure in eggs and nestlings of insectivorous birds downstream of the former PCB production facility near Anniston, Alabama. We also examined concentrations of lead (Pb) and mercury (Hg) (PCB production-associated contaminants) in the avian tissues. Nest boxes targeting Carolina wrens (*Thryothorus ludovicianus*) were placed in four exposure areas along Choccolocco Creek, representing a gradient of contamination, based on soil chemistry data. Additionally, barn swallow (*Hirundo rustica*) eggs and nestlings were collected from under a bridge in the watershed. Collections of wren and swallow eggs and nestlings occurred over two breeding seasons (2014-2015), along with opportunistic sampling of five additional species. Contaminant concentration ranges in Carolina wren eggs were: 1.5-530 μg/g PCBs (wet weight, ww); 40-30,000 pg/g 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxic equivalents (TEQs, ww) from dioxin-like PCBs, polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs); < 0.04-0.51 μg/g Pb (dry weight, dw); and < 0.06-1.88 μg/g Hg (dw). Nestling concentrations were 2–6-fold less for PCBs and 4–10-fold less for TEQs when compared to those in eggs obtained from the exposure areas. Mean concentrations of PCBs and TEQs were elevated in all four exposure areas relative to the reference location (Talladega National Forest). Congener patterns of PCBs will be compared in eggs and nestlings from these two insectivorous passerine species. Exposures of Carolina wren and barn swallow offspring to Pb and Hg were generally below toxicity thresholds, while PCB and TEQ concentrations were among the greatest for any bird species evaluated in North America and were well above toxicity thresholds of PCBs for mortality, development, and reproduction in avian species.

03.07.09 Monitoring and Assessment of Population, Reproductive, and Health Effects in Colonial Waterbirds Breeding at Contaminated Great Lakes Sites in Michigan

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Ongoing immunological, reproductive, and population effects consistent with exposure to polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins, and dibenzofurans (PCDD/Fs) were found in fish-eating birds in the Saginaw River and Bay and River Raisin Areas of Concern (AOCs) and Grand Traverse Bay during 2010-2019. Reference sites were in the lower St. Mary's River (herring gulls and Caspian terns), eastern Lake Superior (terns), and eastern Lake Huron (black-crowned night herons). Relative risk ratios for embryonic nonviability (from both infertility and mortality) in gull embryos were 2-3 fold higher than the reference site in both AOCs and Grand Traverse Bay. Deformities associated with PCBs and 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 of 4 week gull chicks was poor in 7 of 10 years. Numbers of breeding herring gulls decreased significantly in the River Raisin AOC, and breeding Caspian terns, a state-threatened species, declined in the Saginaw River and Bay AOC. The mean 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 in reproductive and immune endpoints in either AOC or Grand Traverse Bay over the study period. Embryonic death increased with time in gull eggs in lower Saginaw Bay, and antibody responses decreased in terns in outer Saginaw Bay.

03.07.10 Continuing Persistence and Biomagnification of DDT and Metabolites in Northern Temperate Fruit Orchard Avian Food Chains

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DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane) was intensively applied from the late 1940s to the 1970s in fruit orchards in the Okanagan valley, British Columbia, Canada, and in the process, contaminated American robin (*Turdus migratorius)* food chains with the parent compound and metabolite, *p,p*'-DDE (1,1-dichloro-2,2-bis(4-chlorophenyl) ethylene). Here, we revisited the same sites twenty-six years after our initial study and: 1) collected soil, earthworms, and American robin eggs from orchards and non-orchard areas; 2) estimated fugacity, biota-soil-accumulation factors (BSAFs), and biomagnification factors (BMFs) on a lipid normalized basis; and 3) characterized the diet and trophic positions of our biota using stable isotope analyses of $\delta^{13}C$ and $\delta^{15}N$. Average *p,p*'-DDE concentrations (soil: 16.1 μg/g organic carbon-lipid equivalent; earthworms: 108 μg/g lipid equivalent; robin eggs: 568 μg/g lipid equivalent) confirmed that contamination is still present at elevated levels similar to the 1990s. Mean fugacities for *p,p*'-DDE were statistically higher in American robin eggs (1168 nPa) than earthworms (223 nPa; $p < 0.001$) and soil (23.3 nPa; $p < 0.001$), suggesting that an increase in thermodynamic potential occurs as *p,p*'-DDE is transferred from prey to predator. The average BSAF and BMF for *p,p*'-DDE was 18.8 μg/g lipid equivalent/OC-lipid equivalent and 7.50, respectively, indicating that *p,p*'-DDE was still biomagnifying in our American robin food web. High *p,p*'-DDE concentrations in American robins in our study (107 μg/g wet weight or 1979 μg/g lipid equivalent) exceeded published levels in migratory birds nesting in orchards, including other thrush species in which reproductive effects were observed. Ongoing elevated concentrations of *p,p*'-DDE in the Okanagan valley may also pose a health risk to local predators and birds of prey, such as *Accipiter* hawks and falcons.

03.07.11 Assessing Halogenated Organic Compounds in California Condors and Their Scavenged Marine Mammal Prey: Implications for Reintroduction

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Since California condors nearly went extinct in 1987, conservation efforts have focused on reintroducing condors to wild habitats where they can build self-sustaining populations. Healthy diets are critical to supporting wild populations, and different reintroduction sites pose varying dietary health risks. Inland habitats are associated with higher risk of lead poisoning, due to the availability of terrestrial game animals, whereas coastal habitats contain lead-free marine mammal carcasses for condors to scavenge. However, recent evidence shows that marine mammal consumption leads to increased exposure of halogenated organic compounds (HOCs), which are associated with eggshell thinning. Using comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GCxGC/MS-TOF), we identified the HOC profiles of inland and coastal condors to detect compounds unique to coastal condors. We then compared coastal condor HOC profiles with existing HOC data from Southern California (CA) marine mammals to confirm that contaminants are being sourced from a marine mammal diet. Finally, we analyzed and compared HOC profiles of cetaceans from the Gulf of California in Baja California, Mexico (Baja cetaceans) with CA cetaceans to determine differences in contaminant loads in each location. Results showed that coastal condors contained significantly more HOCs than inland condors (32 ± 5 vs. 8 ± 2). The most abundant chemical classes in the coastal condors were DDT-related compounds and PCBs. Comparisons of coastal condors and CA marine mammals showed similar profiles, such that >98% of HOCs identified in coastal condors were detected in CA marine mammals, confirming that marine mammal consumption leads to HOC exposure. Baja cetaceans contained significantly fewer HOCs than CA cetaceans (58 ± 19 vs. 124 ± 3) and compounds were less abundant in Baja cetaceans, including a 7-fold reduction in DDT abundance. Focusing reintroduction efforts to Baja California could be advantageous because Baja marine mammals have reduced contaminant loads that might limit HOC exposure and resulting reproductive health effects.

03.07.13 Biotransformation of Dec-604 and Potential Interaction with Thyroid Deiodinase Activity in Highly Flame Retardant Exposed Gulls

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A range of halogenated flame retardants (HFRs) have been recognized as thyroid axis disruptors, especially the ubiquitous polybrominated diphenyl ethers (PBDEs). HFRs are added to numerous consumer products and hence, are released into the environment and have been shown to accumulate at occasionally elevated levels in wild birds, including urban-adapted gulls. The HFR Dechlorane 604 Component B (Dec-604 CB), a putative dehalogenation product of Dec-604, has frequently been determined in tissues of ring-billed gulls *(Larus delawarensis)* breeding in the Montreal area (QC, Canada) and air samples from this region. The metabolic pathways of Dec-604 are yet to be characterized, although the frequent occurrence of Dec-604 CB in gull tissues may suggest enzyme-mediated dehalogenation (bromine removal). Potential dehalogenation enzymes involved in this reaction are the thyroid deiodinases that remove iodine in peripheral tissues of vertebrates. The objective of this study was to investigate in ring-billed gulls the *in vitro* biotransformation of Dec-604 and its interaction with type 1 deiodinase (D1) that catalyzes the formation of triiodothyronine (T_3) and diiodothyronine (T_2) from thyroxine (T_4). We tested the *in vitro* activity of D1 in liver microsomes in the presence of five concentrations of Dec-604 ranging from 0.00086 to 0.086 nM. We also measured HFR concentrations in liver samples of the same individuals. Results showed that total D1 activity in gull liver microsomes was significantly influenced by several concentrations of Dec-604. Moreover, a negative correlation was found between liver HFR concentrations and the rates of T_2 formation in the assay for both sexes and the total D1 activity in males. We also observed that T_2 formation rates in the assays were significantly influenced by the presence of Dec-604 in four of the five concentrations tested. Results also showed that Dec-604 CB was found in the *in vitro* assay at an intermediary concentration of Dec-604 (0.0086 nM); in the majority of the gulls, the increasing activity of D1 in liver microsomes was associated with higher ratios of Dec-604 CB/Dec-604. These results suggest that exposure to Dec-604 may disrupt normal D1 activity in ring-billed gulls. This effect could lead to disruption of the thyroid axis regulation and in turn, circulating thyroid hormone levels in this gull species for which numerous thyroid-related effects have been documented.

03.07.14 Occurrence and Temporal Trends of Industrial Antioxidants and UV Absorbents in the Endangered St. Lawrence Estuary Beluga

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Exposure to contaminants has been identified to be a major stressor that adversely affect the health and recovery of the endangered St. Lawrence Estuary (SLE) beluga (*Delphinapterus leucas*) population. However, the exposure of SLE belugas to many emerging contaminants remains unknown. The objective of this study was to investigate the concentrations and temporal trends (2000-2017) of synthetic phenolic antioxidants (SPAs), secondary aromatic amines (Ar-SAs), benzotriazole UV stabilizers (BZT-UVs), and UV filters (UVFs) in the blubber ($n = 69$) and liver ($n = 81$) of SLE belugas. Results in blubber showed relatively higher detection rates and concentrations of 2,6-di-tert-butylphenol (26DTBP), 2,6-di-tert-butyl-1,4-benzoquinone (BHT-Q), 2-6-di-tert-butyl-4-methylphenol (BHT), 2-hydroxy-4-methoxybenzophenone (BP3) and 2-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethyl butyl) phenol (UV329). The median concentrations (lipid weight basis) were 2.7 ng/g, 77 ng/g, 3.1 ng/g, 6.6 ng/g and 7.2 ng/g for 26DTBP, BHT-Q, BHT, BP3 and UV329, respectively. Female belugas showed significantly higher levels of BHT and BP3 than males. In contrast, the concentrations of UV329 were greater in males compared to females. A positive correlation was found between the concentrations of 26DTBP and BHT-Q, indicating common sources and/or a similar environmental fate of these contaminants. There was no correlation between the concentrations of target contaminants and the ages of belugas. A possible reason is that the predicted biological half-life of these five frequently detected contaminants (e.g., 0.2-10 days) is relatively short. Therefore, the levels of these contaminants may only reflect a short-term accumulation in belugas. For the temporal trends, 26DTBP and BP3 levels in beluga blubber started to increase after 2009. An increasing trend was found for BHT-Q between 2000 and 2005, but there was no change after 2005. In addition, a decreasing trend was found for UV329 between 2000 and 2008, followed by a plateau post-2008. For BHT, there was no clear temporal trend in belugas between 2000-2017. The analysis of liver samples is currently underway. This study establishes a basis for the future monitoring of SPAs, Ar-SAs, BZT-UVs and UVFs in belugas and other marine mammals, although more research is warranted to elucidate the toxicological risks that these emerging contaminants may represent in the aquatic environment.

03.07.17 Factors to Consider When Interpreting Slight Effects on Eggshell Thickness

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Eggshell thickness is one of 15 endpoints evaluated in avian reproduction studies (OCSPP 850.2300, OECD 206). Typically, eggs are reserved for eggshell thickness on predetermined days during the 10 weeks of egg collections. Eggshells are cut open around the equator, contents are discarded, shells are washed and left to dry for ≥48 h prior to measurement with a micrometer to ±0.001 mm. Compared to the quantal endpoints in the test, eggshell thickness has a low coefficient of variation; resulting in effects as low as a 3% thinning from the control being reported as statistically significant. Subsequently, eggshell thickness has been one of the endpoints that most commonly drove avian reproduction study conclusions at Smithers. Even though the organochlorine literature on eggshell thinning indicates population-relevant effects are unlikely below 18% thinning, the 2009 Guidance of EFSA for bird and mammal risk assessments implies that slight eggshell thinning effects, even if there is no subsequent effect on hatchability, cannot be dismissed as not biologically relevant due to potential interspecies differences, the high inter-pair variability and the need to be protective of free-ranging birds on calcium-deficient diets. In order to help determine whether slight eggshell thinning effects can be clearly linked to exposure to a given test material or if this is precluded by other factors, we evaluated the precision and accuracy with which eggshells can be measured. Preliminary results indicate inter-analyst variation is greater than many of the slight effects that have driven study conclusions. While intra-analyst variability appears to be lower, the mean thickness of 45 eggshells still ranged by 5% when shells were measured on 10 occasions during an 8-week period; this was apparently independent of storage time, temperature and humidity. Regulatory agencies are increasingly focused on endocrine- mediated effects, yet there remains uncertainty around the exact mechanisms involved in eggshell thinning. Additionally, an investigation of mechanistic toxicity, molecular, cellular, tissue or organ-level biomarkers is not included in the current avian reproduction study. This engenders a necessity for industry and regulators to understand the precision and accuracy with which eggshell thickness can be measured in the laboratories performing these vertebrate intensive one-shot studies so that slight effects and noise can be differentiated.

03.07.18 Associations Between Organic Contaminants and Vitamins A and E in the Plasma of Great Lakes Pre-Fledgling Double-Crested Cormorants

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Vitamin A plays essential roles in vision, immunity, and development. Vitamin E is an antioxidant and supports immune function. Concentrations of vitamins in tissues can be influenced by diet, tissue type, age, cellular/physiological metabolism, health status, and contaminants. Dioxin-like contaminants such as planar polychlorinated biphenyls (PCBs) have been shown to be associated with reduced vitamin A in wild and experimental animals, including Great Lakes waterbirds. Vitamin E interacts with contaminants like polycyclic aromatic hydrocarbons (PAHs) and dioxins that induce oxidative stress and enzymes. The objectives of this study were to determine 1) whether plasma concentrations of these fat-soluble vitamins (A & 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 2) whether vitamin concentrations were associated with organic contaminants. Across all five Great Lakes, plasma samples were collected from 355 cormorant 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 AOCs or in nearly recovered 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 dioxin toxic equivalents (TEQs) of planar PCBs (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. Vitamin A had moderate negative association with planar PCB TEQs (P< 0.013) and no association with other contaminants, which fit with other studies. 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 (P values>0.7). Causation criteria, including replication (multiple years, multiple 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 a role of contaminants in altering 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.

03.07.19 Associations Between Organic Contaminants and Thiamine in the Plasma of Pre-Fledging Great Lakes Double-Crested Cormorants

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Thiamine (vitamin B1) plays essential roles in energy metabolism, growth, development, immunity, and antioxidant defenses. Tissue concentrations of vitamins can be influenced by tissue type, diet, age, health status, metabolism, and contaminants. Some Great Lakes fish, such as alewives (*Alosa pseudoharengus*), contain high levels of thiaminase, which degrades thiamine. Thiamine deficiency has been identified in salmonids of the Great Lakes and fish-eating birds in other areas. This study's objectives were to determine 1) whether plasma levels of thiamine, thiamine diphosphate (TDP), thiamine monophosphate (TMP), and the sum of all three vitamers (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 2) whether thiamine concentrations were associated with organic contaminants. Plasma samples were collected from 361 cormorant chicks from 16 colonies in 2016 and 15 colonies in 2017 across all the Great Lakes. Combining 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 or in nearly recovered AOCs (t-test P values< 0.0001 except P< 0.0011 for TDP in 2016). Concentrations of contaminants in plasma pooled by site ranged from 4.0–91.9 ng/g for sum PCBs, 0.18–4.6 pg/g for PCB dioxin toxic equivalents (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 thiamine, TDP, TMP, and sum T in both years (Jonckheere trend tests P values< 0.01 except P< 0.032 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 marginal but still significant associations with PCBs. 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 strong to moderate 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 large role in influencing thiamine concentrations in young cormorants. No ecological or dietary factors were apparent as other strong explanations for the differences in vitamin concentrations among colonies.

03.07.20 A Multi-Matrix Metabolomic Evaluation of Ringed Seals: Selecting the Optimal Tissue for Characterizing the Impacts of Contaminant and Climate Related Stressors

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As top predators, ringed seals are particularly vulnerable to elevated concentrations of persistent organic pollutants, such as polychlorinated biphenyls (PCBs). For example, impaired reproduction, endocrine disruption, bone lesions, reduced immune function and tumor incidence have been associated with PCB exposure in ringed seals. PCBs represent the primary contaminant class of concern in Labrador, with significant hotspots associated with two former military radar sites at Saglek and Hopedale. Previous studies found relationships between Labrador coast ringed seal PCB burden and profiles of gene transcripts and metabolites which play an important role in animal health with respect to chemical detoxification, the immune and endocrine systems, and the regulation of growth, development, and metabolism. Furthermore, a shift in Labrador ringed seal foraging and/or feeding ecology have been reported in response to unfavorable ice conditions (e.g., decreased extent of ice coverage and earlier spring breakup), which may, in turn, impact contaminant accumulation. In order to assess the impact of contaminant and climate-related stressors on the health of Labrador ringed seals, a new multi-matrix approach using targeted metabolomics will be presented. Blubber concentrations of several organohalogens (i.e., PCBs, polybrominated diphenyl ethers and chlorinated paraffins), muscle carbon and nitrogen stable isotope profiles (i.e., dietary tracers), as well as profiles of several metabolic classes (e.g., amino acids, biogenic amines, fatty acids, hexoses, acylcarnithines) determined in different matrices (i.e., plasma, liver, and inner and outer blubber) were analysed in ringed seals from Labrador. Preliminary results suggest that plasma and/or liver should be selected for metabolite profiling when studying the influence of diet related changes that may be associated with climate change, while blubber should be used for assessing contaminant associated impacts. This study provides insight into inter-matrix similarities and differences, and assists in the selection and validation of the most optimal matrix for use in assessing the impacts of contaminant and climate related stressors on the health of ringed seal. Results will also help to better understand the global impact of those stressors in marine mammals and may also serve as a reference especially for researchers who only have access to skin/blubber biopsies of marine mammals.

03.07.21 Partial Albinism in Nebraska Barn Swallow Feathers: Elemental Composition, Heavy Metals, and Possible DNA Damage

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North American barn swallows (*Hirundo rustica erythrogaster*) nesting over an agricultural drainage ditch in East-central Nebraska are exhibiting partial albinism and tail streamer asymmetry, an indicator of genetic damage. Facial plumage and tail streamer observations are consistent with a barn swallow population exposed to radiation in Chernobyl, Ukraine. Nebraskan birds were sampled from 2019-2021 and body measures, blood, and feather samples were taken; plumage variation and tail streamers were documented and photographed. Feather samples were analyzed using X-Ray fluorescence spectrometry to identify elemental composition and presence of heavy metals in feathers, as birds often sequester metals in feathers to lessen body burdens. Some, but not all samples, unexpectedly contain arsenic, chromium, and strontium, among other heavy. Chromium and arsenic can be carcinogens to animals and humans, and strontium is highly reactive, rarely naturally-occurring, and usually associated with nuclear fallout. DNA damage analysis, XRF analysis of insect and soil samples, and statistical analyses will occur in summer 2021. Results of this study serve to investigate hypotheses of local, environmental heavy metal contamination and/or the presence of DNA damage in this population. Importantly, results from this study will help to inform the local public who use this waterway as a food source.

03.07.22 Use of Exposure-Response Relationships to Assess Relative Wildlife Risks From Mercury, DDT and TCDD Equivalents in Forage Fish from Contaminated Water Bodies

J. Kubitz, Y.B. Atalay, Cardno

We developed exposure-response relationships for mercury and diclorodiphenyltricloroethane (DDT) from field studies with common loon (*Gavia immer*) and brown pelican (*Pelecanus occidentalis*), respectively. We also extracted an exposure-response relationship for the dioxin-like activity of polychlorinated biphenyls (PCBs) from a laboratory study with mink (*Mustela vison*). All of these equations are based on contaminant concentrations in food items (fish) and reproduction endpoints. These exposure-response relationships can be used to calculate the magnitude of reproductive inhibition to wildlife based on fish tissue samples. Where more than one of the contaminants of interest are present, these exposure-response relationships can be used to compare the relative potential risk or injury to wildlife species from the different chemical stressors. Derivation details and examples of fish tissue interpretations will be presented and discussed.

03.07.24 Establishment of Simultaneous Analysis of Insecticides Using LC-MS/MS and Measuring the Accumulation Amount in Wild Raccoons

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Many negative impacts toward wildlife by insecticide have been reported worldwide. Although no toxic effects were observed using experimental animals at pre-assessment tests, some insecticides were regulated after use because novel toxic effects on wildlife are detected. In this situation, it is necessary to evaluate the toxic effects of insecticides on wild species more accurately. However, only limed toxicity evaluation is done at present risk assessment of insecticides. Especially, toxicological data in terrestrial animals are lacked comparing to aquatic animals. Because the exposure amount or number of insecticides are different by animal species in terrestrial wildlife, we should reveal not only the insecticide residue concentration in environmental samples such as soils, water, and bait organisms but also the accumulation dose in the body of each terrestrial animal. In this study, we focused on raccoons that have been exterminated for invasive alien species in Japan as a terrestrial animal model for insecticide exposure in wild situations. We built a hypothesis that raccoons were exposed to some kinds of insecticides because they inhabit farmland and eat crops like corn or feedstuff of cows in this research area. We established the simultaneous analysis method of currently used insecticides in Japan using LC-MS/MS and measured the accumulation dose in the urine of raccoons. In previous studies on terrestrial animal species, the accumulation doses had been measured mainly in the liver, kidney, lipids, and other organs. In this study, we can detect insecticides more sensitively by using urine samples. In the future, we will measure insecticide accumulation doses in various terrestrial species and perform *in vivo* exposure tests using terrestrial wild animals. By combining the results of accumulation amount and *in vivo* test, we estimate the insecticide exposure doses to terrestrial animals in wild situations.

03.07.25 Evolution by Pesticides: Evidence of Evolution in American Alligators (Alligator mississippiensis) Affected by Endocrine Disrupting Chemicals

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In an increasingly polluted world, contaminants have become a major threat to biological homeostasis and may be eliciting rapid evolutionary responses in exposed wildlife populations. In 1980, Lake Apopka, Florida was the site of a chemical spill releasing thousands of gallons of pesticide mixtures resulting in its classification as a superfund site. These pesticides function as endocrine-disrupting chemicals (EDCs), which are known to affect steroid hormone levels and signaling as estrogen mimics. The exposed native population of American alligators (Alligator mississippiensis) at Lake Apopka have consequently displayed widespread aberrant sexual development, a sharp decline in egg viability, and a drastic decrease in population density following exposure. Surprisingly, the population rebounded a decade later despite the chronic multigenerational persistence of these pesticides in the environment. Despite extensive physiological studies describing the acute and long-standing effects of exposure, the potential role of natural selection in aiding population recovery at this site remains unknown. Notably, a recent study in this contaminated alligator system has shown that exogenous exposure to follicle-stimulating hormone (FSH), a hormone important for reproductive development and function, can lead to only a partial rescue of the Apopka gene transcriptional profiles and phenotypes. Here, I aim to determine if the incomplete FSH transcriptional rescue observed after long-term multigenerational exposure to endocrine-disrupting chemicals at Lake Apopka is the result of selection for physiological and/or developmental resistance.

03.07.27 Microplastics a New Anthropogenic Stress on Marine Turtles

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Microplastics (< 5 mm) are produced by the degradation and fragmentation of macroplastics. This has become a global crisis due to their accelerated increased in the last years and for being present in every ocean in the world. All their sources are anthropogenic; most of them come from terrestrial activities (80%) and from the fishing industry (20%). Different studies have found Microplastics in the water column and in marine sediments. Winds and marine currents cause them to accumulate in some areas that are important for marine organisms, like feeding and nesting areas. The Microplastic physicochemical properties may change when they degrade but, more importantly, they are a transfer vector for other contaminants through the trophic chains. Among them, the persitant organic pollutants (POPs) are important due to their effects on biota, that together with their persistence represent a risk for wild life like marine turtles which are already endangered. The objective of this work was to review the state of the knowledge regarding Microplastics and turtles. The main routes of exposure to plastics for turtles are ingestion and physical contact. Many cases of intestinal obstruction and infections as well as damage to the intestinal track have been reported. There are other effects that have been reported and can be associated to POPs adsorbed in Microplastics like lower nutrient absorption, bioaccumulation in tissues and organs, metabolism alterations, changes in hatching rates and sex ratios, changes in behavioral patterns, loss of the ability to escape from predatos, among others. Migratory species are more vulnerable to these anthropogenic threats. The lack of an adequate strategy of plastic residues management has contributed the their presence in the marine ecosystems. Therefore, it is necessary to take actions to favor a reduction in their generation to conserve marine turtles.

03.07.28 Transcriptomic Biomonitoring of Salmon Smolts Recovering from Exposures to Low Sulfur Marine Diesel Seawater-Accommodated Fractions

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Low sulfur marine diesel (LSMD) is commonly used in aquatic vessels and is a frequent source of contamination from oil spills in the high traffic marine environment of the Pacific Northwest. Despite physical remediation efforts, water-soluble components including polycyclic aromatic hydrocarbons (PAHs) can persist, thereby potentially affecting proximal aquatic biota. The coastal cold-water areas encompass critical habitats for many aquatic organisms including salmonid species that are vital to local fisheries. Rapid and sensitive monitoring of the environmental effects of oil spills has considerable ecological and economic implications, especially for subsequent remediation efforts. To establish sensitive, non-lethal bioindicators for monitoring oil spills and remediation, the transcriptomic responses of juvenile coho salmon smolts (*Onchorhynchus kisutch*) exposed to 100 mg/L LSMD seawater accommodated fractions (seaWAF) with subsequent depuration in clean seawater were investigated. Smolts were collected after a 96-hour seaWAF exposure and at 1-, 2-, 4-, and 8-days post-depuration. Liver and caudal fin were dissected from 40 smolts at each time point from seaWAF-exposed and control seawater conditions for a total of 80 smolts per collection period (total n = 400 smolts). Abundance variations in the PAH-sensitive transcript 3-methylcholanthrene responsive cytochrome P450-1a (*cyp1a*) were assessed by quantitative polymerase chain reaction (qPCR) analysis. Having previously demonstrated that seaWAF exposure dramatically increased *cyp1a* in both tissues, we further assessed whether *cyp1a* could be employed to monitor remediation. Our results show that *cyp1a* transcript amounts decreased within one day of depuration in both the liver and caudal fin of sex genotyped females and males. Furthermore, the caudal fin of females at depuration day 1 showed complete recovery of *cyp1a* abundance, while the male caudal fin and female and male liver tissues recovered after two days. The recovery was maintained up to day 8. The robustness of *cyp1a* decreases provides strong support for the application of *cyp1a* transcriptomic assessments as a biomonitoring measure of contaminant and remediation activities. Additionally, sex-dependent differences in recovery should be comprehensively explored on a transcriptome-wide basis. The current study demonstrates the potential value in using the caudal fin as a reliable, sensitive, and non-lethal sampling method.

03.08 Terrestrial Toxicology, Ecology and Stress Response

03.08.01 Screening-Level Pollinator Risk Assessment for Trisiloxane Polyether Surfactants

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The USEPA, PMRA, and CDPR (2014) pollinator risk assessment process is primarily focused on active ingredients of agricultural chemicals. However, recent scientific interest is growing reqarding the potential impact of the inert ingredients of end-use agricultural products. One class of these ‘inerts’ is trisiloxanes, which represent superspreader surfactants. Superspreaders have unique properties which significantly reduce the surface tension of water to promote a rapid spreading of aqueous solutions on the surfaces of leaves. Application of the screening-level risk assessment process for these chemicals poses the challenges of conservatively quantifying exposure and determination of toxicological endpoints. This presentation addresses methodologies employed to conservatively quantify exposure in order to apply the screening-level pollinator risk assessment framework for three trisiloxane polyether surfactants. Two methodologies to estimate exposure are employed here, including estimation of maximum pollinator-relevant residues from magnitude of residues studies, as well as queried data analyses from the CDPR Pesticide Use Registry (PUR) database, which has included adjuvant use entries since its inception. The results from an effects characterization for larval and adult stage honeybees (*Apis mellifera*) with the exposure quantification to run the BeeREX model for evaluation of Tier 1 screening-level risk. The estimated environmental concentrations (EECs) and effects endpoints are used to calculate risk quotients for comparison to the defined levels of concern for pollinator risk assessment. Overall screening-level risk assessment findings will be presented along with an uncertainty analysis.

03.08.02 Assessing Monarch Caterpillar (Danaus plexippus L.) Survival from Exposure to Fungicide-Insecticide Combinations

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The intensively cultivated Midwestern U.S. is a crucial breeding ground for the monarch butterfly (*Danaus plexippus* L.). Monarch butterfly larval host plants, *Asclepias* spp., frequently occur near agricultural fields and pesticide residues are often present on these milkweeds. Therefore, monarch caterpillars present on *Asclepias* spp. close to agricultural fields are likely exposed to multiple pesticides. Previous studies with the honey bee, *Apis mellifera*, indicated synergistic interactions between demethylation-inhibiting (DMI) fungicides and pyrethroid, anthranilic diamide, and neonicotinoid insecticides, leading to higher mortality. The increased mortality caused by DMI fungicides and insecticide combinations is hypothesized to occur via the inhibition of cytochrome P450 detoxification enzymes by DMI fungicides. Interactions between DMI fungicides and insecticides in monarch caterpillars have not yet been studied. This research focuses on evaluating the interactions between a prominently used DMI fungicide, propiconazole, and the insecticides bifenthrin, chlorpyrifos, and thiamethoxam in monarch caterpillars. This study evaluated 3rd-instar monarchs orally exposed to combinations of propiconazole and each insecticide via incorporation into an artificial diet. Mortality, behavior, and morphology were recorded daily for 96 hours. After the 96-hour exposure, the caterpillars were observed until pupation and adult emergence to record lethal and sublethal effects. Pesticide exposures were based on worst-case scenarios for spray drift in agricultural ditches via modeling pesticide deposition at 0, 5, and 10 meters from application fields using the high spray label rates of formulated products with the AgDRIFT program. The results from this research demonstrate plausible impacts of exposure to fungicide-insecticide combinations on monarch caterpillars developing close to agricultural fields.

03.08.03 Assessing the Impacts of Trace Metal Contamination on the Viability, Biomass, and Behavior of the Earthworm (Eisenia fetida) Using an Agar Medium

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Trace metals such as arsenic (As) and lead (Pb) have been found as components of acid-mine drainage emanating from deep mine sites and tailings. As these metals are water-soluble, they can enter both aquatic and terrestrial environments. The earthworm, *Eisenia fetida*, is commonly used in toxicology studies as an indicator species due to their sensitivity to soil conditions and ease of maintenance under laboratory conditions. *E. fetida* biomass and behavior can be used as an indicator of health to reflect the conditions of their habitat. While behavior is typically tested by measuring soil aversion based on dispersion or reactivity to a probe test, unstimulated behavior of earthworm as a measurement of mobility have not been explored. Additionally, agar is a simple source of nutrients and a viable long-term habitat medium for earthworms that allows for the possibility of testing the isolated impacts of trace metals on earthworm biomass and mobility. Thus, this study was designed to investigate the effects of two neurotoxic trace metals, As and Pb, at environmentally relevant concentrations on viability, body mass, and behavior of *E. fetida* using an artificial agar medium. Worms will be exposed to agar spiked with either 0, 10, 25, 50 or 100 mg As/kg wet agar or 0, 40, 100, 200, or 400 mg Pb/kg wet agar for 14 days. The biomass of each worm was assessed on days 0, 7, and 14 and differences in locomotor behaviors (average speed, mobile average speed, average acceleration, total distance traveled, frozen events, and frozen time) quantified using ToxTrac (v. 2.91) pre and post experiment. While this study is ongoing, we expect to observe a dose-dependent decrease in biomass and mobility in earthworms with both metals. Additionally, we expect more severe impacts in the As treatments compared to the Pb treatments. This study will provide a greater understanding of the impacts of trace metals on earthworms using automated behavior software and the suitability of agar as a medium for earthworm exposure tests.

03.08.05 Evaluating the Interaction Between Oxidative Stress Management and Insecticide Susceptibility in Grape Berry Moth (Endopiza viteana)

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The economy of the Mid-Atlantic United States is driven largely by agricultural activity, including the viticulture industry. One issue that the grape industry faces regularly is the control of invertebrate pest species: in the Lake Erie wine belt, one of the most prevalent pests is the native grape berry moth (GBM; *Endopiza viteana*). The efficacy of insecticide treatments can be significantly reduced by the evolution of insecticide resistance. The physiological basis of insecticide resistance is often complex and can be influenced by other seemingly unrelated biochemical processes (e.g., oxidative stress management). Although oxidative stress is known to induce the activity of enzymes that are involved in xenobiotic metabolism, the direct influence of this process on insecticide susceptibility is unknown. This study explores the relationship between oxidative stress and susceptibility to a common-use pyrethroid insecticide (bifenthrin). Using local GBM populations as a model, the *in vitro* activity of glutathione-S-transferase (GST) will be quantified before and after the induction of oxidative stress (via paraquat exposure). Following these assays, acute toxicity tests will be performed on first-instar larvae to determine the relationship between GST specific activity and bifenthrin sensitivity. The information gathered from this study will improve our understanding of the interplay between exogenous and endogenous stressors in the context of insecticide resistance.

03.08.06 Evaluation of In Vitro Toxicity of Organophosphate Insecticide, Dimethoate on Honeybee Larvae (Apis mellifera L.) by Acute and Chronic Exposure

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The Honeybee, *Apis mellifera* L. is present worldwide. It plays a very important role as pollinator for agricultural crops and for wild plants. Loss of honeybee colonies and disorders are observed in many countries. The current situation threatens the continuity of pollinators role and the balance in nature. Multiple factors have been attributed to this decline, which includes parasites, pathogens, poor nutrition, queen failure, habitat loss and migratory stress and extensive use of pesticides on crops. Among these factors, the potential impact of pesticides, particularly those applied in agricultural settings, are of particular interest to us. In the current research, organophosphate insecticide, dimethoate (DMT) has been used. The aim of the current research is to evaluate the toxicity on honeybee larvae (*Apis mellifera* L.) with acute and chronic exposure of DMT. Protein and energy are required for honeybee growth and development. This requirement is fulfilled with pollen and nectar stored in the hive which is carried by worker honeybees. During foraging, honeybee carries pesticides in the hive which exposed to nurse bees to feed the larvae. In the recent work, we exposed DMT to honeybee larvae (*in vitro*) in single (acute) and repeated exposure (chronic). Honeybee larvae were exposed with DMT at the dose levels of 0.0 (control), 0.13, 0.25, 0.50, 1.00, 2.00, and 4.00 µg/larvae, in single exposure and at the dose levels of 0.0 (control), 0.4, 1.1, 3.3, 10.0, and 30.0 mg a.i./kg diet, in repeated exposure. Larvae were observed for mortality, behavioural symptoms, morphological differences and adverse effects after emergence. A significant decrease in survival was observed between 0.13 and 4.00 µg/larvae in single exposure and between 1.1 and 30.0 mg a.i./kg diet. These results represent the assessment of the effects of DMT in honeybee larvae and should contribute to studies on honeybee colony decline. Overall, our findings are valuable for assessing the acute and chronic toxicity of developing honeybee.

03.08.07 Examining the Role of Phase III Metabolism in Insecticide Susceptibility of Grape Berry Moth (Endopiza viteana)

T.A. Long, Penn State Behrend / Biology; A.M. Simpson, Penn State Erie, The Behrend College / Biology; S.A. Nutile, Penn State Behrend / Biology

The economy of northwestern Pennsylvania relies heavily on the production of the viticulture industry. To ensure adequate harvests, viticulturists utilize synthetic insecticides to control pest numbers and prevent the destruction of crops. Due to the frequent use of these chemicals, there is concern that local populations of the grape pest, grape berry moth (GBM; *Endopiza viteana*), have developed resistance to commonly used insecticides. Although pesticide resistance in *E. viteana* is well-documented, the exact mechanisms of resistance are unknown. Therefore, the objective of the current study is to explore the role of ATP-Binding Cassette (ABC) transporters in the development of resistance in local populations of *E. viteana*. ABC transporters are a mechanism of Phase III metabolism that utilizes active transport to translocate potentially harmful metabolites out of a cell. To address this objective, acute toxicity tests will be conducted using first-instar larvae (harvested from local vineyards and a reference site) and will assay baseline sensitivity to common-use insecticides (imidacloprid and α-cypermethrin). Once the baseline toxicity is known, these tests will be repeated following exposure to known ABC inhibitors (verapamil, cyclosporin-A). The role of ABC transporters in insecticide resistance is not well-documented; therefore, this study will provide potentially valuable information that may aid pest control efforts in Pennsylvania and abroad.

03.08.08 Oxidative Stress in the Seaside Sparrow (Ammospiza maritima) Following the Deepwater Horizon Oil Spill

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The Deepwater Horizon (DWH) oil spill introduced massive amounts of oil into Louisiana saltmarshes. Seaside Sparrows (*Ammospiza maritima*) incorporated petrogenic carbon into their tissue and showed extensive changes in gene expression, including *cyp1a*, a gene involved in metabolizing polycyclic aromatic hydrocarbons (PAHs). PAH metabolism generates reactive oxygen species, inducing oxidative stress when antioxidant defenses are overwhelmed. Our study aimed to determine whether DWH oil exposure increased oxidative stress in Seaside Sparrows. We measured plasma reactive oxygen metabolites (ROMs) and antioxidant capacity in sparrows from oiled and control sites (2013-2014). In cardiac tissue samples (2013-2015), we measured total glutathione (GSH) and oxidized glutathione (GSSG) and analyzed total protein damage using Western blot. To account for variation in exposure among site locations, we also used sediment concentrations of PAHs in the site of capture. In 2013, but not in 2014, sparrows from oiled sites had higher circulating antioxidants. Throughout the study period, the concentration of ROMs increased with increasing sediment PAHs in sparrows from control sites, but not in sparrows from oiled sites. The GSSG:GSH ratio in the heart, indicative of intracellular redox potential, decreased with sediment PAHs. Protein damage in the heart tissue of oil-exposed sparrows was higher in 2015, but not in any other year. Contrary to the hypothesis of induction of oxidative stress, these results generally document the maintenance of oxidative status in birds from oiled sites. The higher plasma antioxidant capacity of birds from oiled sites and the stable ROMs at the increase of sediment PAHs suggest mobilization or upregulation of their antioxidant defenses in 2013. Mitigation of oxidative stress is also supported by the decrease in heart GSSG:GSH ratio and no difference in protein damage with increasing sediment PAHs. Yet, variation across years suggests other environmental stressors might eclipse or compound the toxic effects of PAHs. Our data confirm the importance of utilizing multiple biomarkers to assess oxidative stress, those in the circulation and other organs. Future studies will need to investigate to what degree this maintenance was due to antioxidant synthesis or mobilization, and any life-history costs of such metabolic changes.

03.08.09 Physiological Responses to Urbanization in Song Sparrows (Melospize melodia)

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Urbanization can rapidly change the landscape and present novel stressors to animals inhabiting urban environments. For instance, urban birds must cope with altered prey communities and nutritional resources, as well as exposure to contaminants and anthropogenic sound and light. While several studies have compared the physiological differences between urban and rural birds, these studies have yielded conflicting results. Furthermore, most of these studies have examined birds in highly urbanized metropolitan habitats, and the impact of moderate urbanization on avian physiology remains unclear. In this study, we examined physiological differences between rural and urban male song sparrows (<i>*Melospiza melodia*</i>) from in and around Blacksburg, VA, USA. Specifically, we measured packed cell volume (PCV), hemoglobin concentration, circulating glutathione concentration, oxidative damage, and body condition. Additionally, we measured white blood cell differential and bacterial killing ability (BKA) of plasma to assess immune function. Song sparrows inhabiting urban environments exhibited lower hemoglobin, but higher body condition and furcular fat scores. Additionally, urban birds had higher heterophil counts and lower lymphocyte counts, but there were no differences in heterophil:lymphocyte ratio or BKA between urban and rural birds. PCV, glutathione concentrations, and oxidative damage did not differ between urban and rural sparrows. These data suggest sparrows from rural and urban habitats exhibit phenotypic differences in energy storage and metabolic demand, which may be related to behavior differences previously observed in sparrows from these populations. Furthermore, this study highlights the need for measuring multiple markers of physiology to accurately assess the effects of urbanization on birds.

03.20 Late Breaking Science: Terrestrial Toxicology, Ecology and Stress Response

03.20.01 Analysis of Small Polyamide and Polyethylene Microplastic Particles in Rat Feed and Feces and its Effect on the Expression of Inflammatory Proteins in Rats

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Background:Microplastic particles (MP) are ubiquitous in the environment and ingested by humans. Little is known about the absorption, metabolism and effect of microplastic particles in humans or mammalian model organisms. One of the major obstacles of the MP identification is the limited availability of suitable protocols to extract and preserve MP from different sample matrices.Objective: The objective of our study was to establish an efficient method for the quantification of MPs in the complex sample matrices of rat feed and feces. In addition, we aimed to investigate the effect of PE and PA on the expression of inflammatory proteins in rats after exposing them with PE or PA through their feed. Methods: In this study, 24 Wistar rats were fed with feed containing virgin MPs (0.1% PA; mean particle size 15-20 µm) or PE (mean particle size 40-48 µm), or a mixture of PA/PE (0.05 % PA/0.05 % PE), or a control diet free from added MPs, for five weeks. After euthanisation, feces were collected directly from the colon to avoid possible contamination and part of the duodenum was saved for qPCR analysis of inflammatory proteins. MP particles were identified by Pyrolysis-gas chromatography-Orbitrap™ mass spectrometry in feces of ratsResults:In this study, an optimized, rapid and efficient protocol for the digestion of rats' feces and feed was developed. It consists of a combination of mild acidic oxidative (H_2O_2 15%: HNO_3 5%) and alkaline treatment (10 % KOH) followed by enzymatic digestion (Viscozyme®L). PA and PE were quantified in feces of the PA and PE exposure groups. Neither PA nor PE was detected in feces from control rats. The method had good recovery rates for the same MPs as used for the feeding experiment. In addition, gene expression of inflammatory proteins TNF-α, IL-10 and C3 in rat duodenum were measured and showed no significant changes. Conclusions: Analysis of feces may serve as direct evidence of acute MPs ingestion, thus can likely be used to estimate the burden of oral MP exposure in mammalians and oral feeding experiments in rodents or other laboratory animals. These results proved that our proposed digestion protocol is a reliable approach to extract and detect MPs from the feed and feces of rats. The MPs chosen in this study in the mentioned conditions did not cause inflammation.

03.20.02 Characterization of Naphthalene Degrading Acinetobacter Isolates

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Polycyclic aromatic hydrocarbons (PAHs) are a class of chemicals that occur naturally in coal and crude oil. They also are produced as a result of human activities such as burning of coal, oil, gas, wood, garbage, and tobacco. PAHs are highly stable, can bioaccumulate in environmental compartments and can cause serious health impacts in humans. Many bacteria have been found to metabolize PAHs. Bioremediation is an environmentally friendly and inexpensive strategy that removes toxic PAHs *in situ,* for instance after an oil spill. In this study, a variety of naphthalene-degrading bacteria were isolated from a naphthalene enrichment culture established from field collected soil. Naphthalene is the simplest PAH consisting of two fused benzene rings and is often used as a model compound for PAH degradation. Thirty-two isolates were identified by sequencing of the 16S rRNA gene including: *Acinetobacter seifertii* (25), *A. pittii* (4), *A. baumannii* (1), *Rhodococcus agglutinans* (1) and *Salmonella enterica* (1). Degraders were identified by their ability to grow on naphthalene as a sole source of carbon and energy and by the production of indigo from indole, a characteristic of naphthalene dioxygenase, the enzyme catalyzing the first step in PAH biodegradation. Since little is known about PAH degrading *Acinetobacter*, we selected ten isolates for further study. Growth assays were performed on naphthalene, biphenyl and phenanthrene and revealed that all supported growth with naphthalene yielding the highest growth. Emulsification (E_{24}) and Bacterial Adhesion to Hydrocarbons assays were conducted to screen for biosurfactant producing isolates and all were found to display a high adherence capacity to hydrophobic compounds. The genomes of the selected isolates are currently being sequenced which will allow for the identification and PAH degradation genes. Overall, the *Acinetobacter* isolates are showing promising PAH bioremediation capabilities which is surprising, as members of this genus are better known as opportunistic human pathogens.

03.20.03 Estimating the Range Shift of Beetle Occurrence Using Species Distribution Modeling - an Application in the Climate-Change Perspective

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With a changing climate, many species including agricultural pests are likely to shift their geographical ranges. This has important implications for pest management and crop yields. How could this shift look like for major pests? To get an idea for a specific example, we analyze the occurrence data of the spotted cucumber beetle (SCB), a significant pest to cucurbit crops in the US. The purpose is to (i) explore the presence of suitable habitat in the USA and (ii) project the habitat suitability to the future, considering different climate change scenarios. The work is performed using a flexible and open platform for modeling species niches and distributions. To build a species distribution model, data on species occurrence (locations where the species was observed) and environmental conditions relevant to the species across the US have to be retrieved and processed. For our analysis, we used the dataset of the SCB occurrences from the Global Biodiversity Information Facility and the environmental data from WorldClim. After processing these datasets, we performed a partition of occurrence data to test the accuracy of the predictive model. Subsequently, we ran the species distribution model which is based on a machine learning algorithm that estimates the species response to the environment. Model outputs show the relative abundance or probability of occurrence over space (the USA) and time (projections to 2070). We tested two different scenarios of climate change using the intermediate RCP4.5 scenario and the worst case RCP 8.5 scenario. Results suggest that there will be areas of estimated range loss of SCB (Southeast US, Southern Texas) and areas of estimated range expansion (Northeast, Northern parts of the Midwest, Eastern regions of CA, and the Pacific North West) in both climate scenarios, suggesting that the species could become a major pest in regions where it is currently absent or occurring at low abundances.

04.01 Assessing Risk Exposure in the Groundwater - Surface Water Transition Zone

04.01.01 Lessons Learned in Identifying Dominant Stressors on Benthic Communities at the Groundwater-Surface Water Interface

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A synoptic assessment utilizing physical, chemical, biological and toxicity characterization tools is required to evaluate the potential effects of groundwater upwelling on benthic organisms and communities. Groundwater-surface water interface (GSI) characterizations were conducted to evaluate the nature and extent of contamination, potential upwelling and interaction of site groundwater with surface water in an adjacent river, and any potential adverse effects on resident benthic macroinvertebrate communities. Chloride, pH, total dissolved solids, and ammonia were identified as contaminants of potential concern (COPCs). The phased assessment focused on accurately characterizing stressor exposures linked to biological effects. Near-bottom ammonia concentrations and porewater pH at one station were elevated and approached published toxicity thresholds. Benthic community structure observed at most sampling stations was comparable to reference conditions. However, lower diversity and a moderately degraded to degraded community was found at a station exhibiting elevated ammonia and pH. A moderately degraded benthic community was also observed at another nearby station, where elevated COPCs were not observed but where cyanobacteria blooms occurred periodically. Stressor-tolerant species including gastropods and isopods were abundant at the two impacted stations, but pollution-sensitive amphipods were also abundant. Monitoring and *in situ* toxicity testing (*Hyalella azteca* and *Daphnia magna*) revealed very low overnight dissolved oxygen levels (0-1 mg/L) and toxicity occurred at both impacted stations. Results suggested elevated ammonia and porewater pH (perhaps combined with low DO) were likely stressors at one station, while (naturally) low DO alone was likely responsible for observed mortality at the other station. The abundance of amphipods on organic debris resting on the sediment surface at these stations indicated that any effects of COPCs or low DO were restricted to the sediment (i.e., porewater) and did not affect organisms found on or above the sediment surface. Results from this study illustrate the importance of using a strategic, weight-of-evidence approach to accurately link stressor exposure to ecological risk.

04.01.02 Empirical Bioavailability Modeling of Aquatic Fluoride Toxicity - Considerations for Site-Specific Protective Values

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Bioavailability models are important tools to help quantify appropriate protective values for aquatic life based on site- or media-specific conditions known to affect exposure. The application of bioavailability models is of particular importance when considering the groundwater-surface water interface and sediment-pore water transition zone, areas where strong physical and biogeochemical gradients can occur. Although substantial advances have been made to understand and characterize the toxicity of metals using bioavailability models, few studies exist that explore the application of empirical bioavailability models for understanding ecotoxicity of inorganic anions to aquatic organisms. Moreover, without accounting for site-specific biogeochemical factors that are known to modify or ameliorate toxicity, estimates of risk associated with exposure conditions may be over or underestimated leading to greater uncertainty when making remedy management decisions. We conducted a meta-analysis of available aquatic toxicity literature for fluoride to evaluate the utility of site-specific geochemical cofactors such as, hardness, alkalinity, and chloride in freshwater taxa empirical bioavailability models and are presenting a refined application of such models to address a more robust assessment of site-specific exposure conditions. The resulting optimal multiple linear regression (MLR) model predicting acute fluoride toxicity to the invertebrate *Hyalella azteca* included all three cofactors (Observed versus Predicted LC_{50}, $R^2 = 0.89$) and the optimal model predicting toxicity to the fish *Oncorhynchus mykiss* included alkalinity and hardness ($R^2 = 0.41$). The assessment of the model performance indicated that there was increased uncertainty at low (< 20 mg/L) chloride concentrations due to *H. azteca* sensitivity. At >20 mg/L chloride, the preliminary final acute values (FAVs) for fluoride were within one order of magnitude and ranged from approximately 18 to 64 mg/L, depending on water chemistry. Due to limited toxicity data, chronic bioavailability models were not developed and final chronic values (FCV) were instead derived using an acute to chronic ratio (ACR) approach. Accounting for cofactors, the median ACR for fish and invertebrate taxa (n=7) was 4.6. Through careful review, our analysis showed that existing promulgated protective values for fluoride are likely to be overly conservative. In addition, this assessment demonstrates the need to consider bioavailability models when evaluating exposure conditions in dynamic settings, such as the groundwater-surface water transition zone. This assessment highlights the need to expand multivariate empirical bioavailability modeling to include inorganic anions, particularly for fluoride, to ensure that protective values are commensurate with site- or media-specific conditions.

04.01.03 The Application of a Monoclonal Antibody-Based Biosensor for Rapid Assessment of PAH Concentrations in Porewater to Evaluate Transport and Potential Effects at Field Sites

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Polycyclic aromatic hydrocarbons (PAHs) accumulate in sediments, are potentially toxic and carcinogenic and thus are often the target for remediation efforts. They can occur in high concentrations in groundwater that can be a significant source for contamination to aquatic environments. A biosensor that quantifies total PAHs to sub-ppb concentrations in small volume (5-10 mL) aqueous samples in minutes was developed allowing analysis at spatial and temporal scales not possible by GC-MS methods. Biosensor measurements were highly correlated to GC-MS analysis and were also shown to agree with toxicity predictions made by passive sampling techniques. Biosensor measured porewater concentrations were better predictors of amphipod toxicity than the whole sediment PAH concentrations that are often used for regulatory evaluation of sediment remediation effectiveness. Combined with seepage meter sampling, the biosensor provided rapid evaluation of the mechanisms controlling PAH transport and flux at creosote-contaminated sites saving costs and time over traditional methods. Sediment porewater samples at a site under evaluation for a remediation plan were analyzed by biosensor and the results reported to the field crew within twenty-four hours to help guide sediment collection the next day. Used as a rapid screening tool, the biosensor analyses can help evaluate the spatial and temporal variations in PAH concentrations to better understand the sources, flux and potential toxicity of PAHs at contaminated sediment sites. This work was supported by the NIEHS Superfund Research Program grant RO1ES024245.

04.01.04 Evaluating the Seasonal Variability of Groundwater Chloride Inputs to Urban Streams

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Increasing salinization of surface waters due to excessive chloride inputs is a growing threat to aquatic ecosystems. An important source of chloride in temperate climates is road salt, used as a de-icing agent on roadways, sidewalks, and parking lots. Road salt can travel to freshwater streams through direct surface runoff, stormwater drainage, and via infiltration and groundwater transport. Most prior research has focused on the direct runoff pathway and resulting increased surface water concentrations, while few studies have evaluated the groundwater pathway and its potential impact on benthic zone chloride concentrations. The objective of this study is to evaluate the temporal variability of chloride exposure from road-salt impacted groundwater to endo- and epibenthic zones of urban streams. This objective was addressed by conducting field work in two urban streams located adjacent to major arterial roadways in London, Ontario. The porewater streambed chloride concentrations, representing endobenthic exposure to the discharging groundwater, were measured along ~100 m stream sections monthly starting November 2020. These concentrations were typically > 120 mg/L, the long-term freshwater aquatic life guideline for chloride in Canada, during all sampling events. These data also showed substantial spatial variation in chloride concentrations, with highs > 5000 mg/L generally nearest the roadways and only 20 m away from areas at ~ 150 mg/L. There are initial indications of a seasonal change to the porewater spatial pattern. Novel electrical resistivity tomography (ERT) surveys are also being performed at one of the stream sites every month to permit time-lapse monitoring of changes in electrical resistivity (reflecting changes in chloride concentrations) in the groundwater below the streambed between sampling events. Finally, streambed temperature and electrical conductivity mapping will be used to identify groundwater discharge patterns and assess changes impacting the epibenthic zone exposure. Future work will incorporate streambed mussel surveys, to investigate if the groundwater chloride is inhibiting these benthic-dwelling organisms (many of which are species at risk). The study findings are needed to inform road salt usage and management programs and policy across North America, as well as inform ecological management and restoration initiatives for urban streams.

04.02 Chemicals in Domestic Wastewater and Landfill Leachate: Occurrence, Fate, and Use as Tracers

04.02.01 Artificial Sweeteners for Tracing Inputs of Wastewater-Affected Groundwater to Surface Waters

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Over the past decade, artificial sweeteners (ASs) have been increasingly used by researchers as a tracer to investigate wastewater inputs to surface water and groundwater environments. Many ASs occur in ionic form, allowing them to travel well through groundwater systems. Therefore, their detection in groundwater discharging to a surface water body can indicate wastewater inputs through this subsurface pathway. With those AS may come wastewater sourced nutrients, with implications for eutrophication and harmful algal blooms, and various hazardous chemicals (e.g., pharmaceuticals), with implications for human and ecological health. This area of study has been a key focus of our research group over the past decade and continuing today, with surface water and groundwater samples analyzed by ion chromatography, triple-quadrupole tandem mass-spectrometry (negative electrospray ionization mode) for the artificial sweeteners acesulfame, cyclamate, saccharin and sucralose. This presentation will demonstrate a range of applications of this suite of ASs for assessing wastewater contaminant exposure and inputs to surface water systems. Cases will include specific groundwater plume studies to subwatershed scale assessments, in both rural and urban settings.

04.02.02 Pharmaceuticals in Groundwater Under the Influence of Onsite Septic Systems

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Contaminants of Emerging Concern (CECs), as a class of chemicals and potential biological stressors, are difficult to measure, and can be linked to many ongoing and historic sources. One challenge with source identification and mitigation is that in many instances multiple sources contribute to contaminant loading to the aquatic system. Research has identified municipal wastewater-treatment discharges (e.g. aqueous effluents, biosolids) as important sources of CECs to the environment. Although more than 20% of households across the United States use private onsite septic systems, yet understanding and characterizing environmental exposures and corresponding environmental effects derived from such sources are lacking. Groundwater is an important resource to the Cape Cod region of Massachusetts. Cape Cod's fifteen towns rely on a sole-source aquifer for drinking water and groundwater is the primary receiver of wastewater disposal for both on-site and municipal wastewater treatment. To better manage wastewater input to the aquifer and to improve groundwater quality, over 1,400 homes in the town of Falmouth were connected to the municipal sewer system beginning in 2016. This conversion from onsite septic to a municipal sewer system provided a unique opportunity to observe potential changes occurring in groundwater quality in response to the change in the CEC source term. The results of this study will provide important information to other areas where aquifers are overlain by high densities of onsite septic systems. Groundwater samples were analyzed for over 100 chemical and microbial CECs. Maximum observed concentrations of target CECs were often found at similar (e.g. buproprion, cotinine, tramadol) or even higher (e.g., carbamazepine, fluconazole, sulfamethoxazole, sucralose) concentrations compared to that found in municipal wastewater effluent. These results clearly document onsite septic systems as another important environmental pathway for CECs.

04.02.03 Volatile Per- and Polyfluoroalkyl Substances (PFAS) Analysis in Landfill Gas Using Thermal Desorption Coupled With Gas Chromatography Mass Spectrometry

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Over half of the municipal solid waste generated in the United States is disposed in landfills. Some of this waste includes consumer products, which are known to contain precursor to per- and polyfluoroalkyl substances (PFAS), including volatile PFAS, such as fluorotelomer alcohols (FTOH), perfluoroalkane sulfonamides (FASA), and perfluoroalkane sulfonamido ethanols (FASE). While there are reports of non-volatile PFAS in landfill leachate, little is known about the occurrence of volatile PFAS in landfill gas. Data exist for volatile PFAS in ambient air near (downgradient) of landfills in North America, Europe, and Asia. In this work, thermal desorption (TD) tubes were used to collect volatile PFAS in landfill gas in the southeastern region of the United States. Once collected, TD tubes were desorbed and volatile PFAS were analyzed using gas chromatography coupled with mass spectrometry (GC-MS). A safe sampling volume of 350 mL of landfill gas was collected at a flow rate of 100 mL/min. The TD-GC-MS method was modified from the U.S. EPA

TO-17. Whole method recovery ranged from 50-130%. Preliminary data from landfills in the southeastern region of the United States indicates that FTOHs dominate the profile of volatile PFAS in landfill gas, with concentrations two-to-three orders of magnitude higher than other volatile PFAS as well as FTOHs in ambient air in landfills previously reported. Other volatile PFAS, including FASAs, fluorotelomer olefins, and fluorotelomer acrylates were also quantified in landfill gas. Initial comparison between old and new cells indicated higher concentrations of 6:2-FTOH in new cell, while concentrations of 8:2- and 10:2-FTOHs were comparable between new and old cells. This work highlights the application of TD-GC-MS to collect and analyze volatile PFAS in landfill gas and indicates that volatile PFAS from consumer products may partition into landfill gas. The results show that uncollected landfill gas is a source of PFAS in the atmosphere and also emphasize the need for studies on the fate of PFAS in landfill gas that is collected and either burned in a flare, engine, or boiler.

04.02.04 Seasonal Trends of Antibiotic Resistance in Oregon Wastewater Treatment Plants

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With the overuse and disposal of antibiotics in hospitals, agriculture, and residentials, wastewater treatment plants are considered one of the main sources and reservoirs of antimicrobial resistance (AMR). To understand proliferation and persistence trends of antibiotic resistance in wastewater treatment plants, this study investigated the prevalence of eight different phenotypes of AMR and multi-drug resistance *E. coli* in 17 wastewater treatment plants across Oregon in winter and summer of 2019 and 2020. Overall, 1187 *E. coli* colonies were isolated from influent, secondary effluent, final effluent, and biosolids. 31.8% of all collected isolates exhibited resistance to at least one antibiotic, and 8.7% carried multi-drug resistance phenotypes, of which 16.2% harbored resistance to more than five classes of antibiotics. From a total 246 sampling incidences, 23 demonstrated multiple antibiotic resistance index above 0.2, suggesting high risk of antibiotic contamination. Significantly higher multiple antibiotic resistance index and prevalence of ciprofloxacin-resistant *E. coli* was observed in biosolids. Highest AMR rates amongst collected *E. coli* isolates were observed for ampicillin (18.4%) followed by streptomycin (13.8%) and tetracycline (13.6%). Additionally, resistance to trimethoprim-sulfamethoxazole showed significant associations with the occurrence of ampicillin, tetracycline, and streptomycin phenotypes ($p < 0.01$). When comparing different phenotypes of AMR between winter and summer in the influent, significantly higher prevalence of ciprofloxacin-resistant *E. coli* was observed in summer ($p < 0.01$), suggesting the seasonality of different phenotypes. Findings suggest that the removal efficiency of AMR *E. coli* in wastewater treatment plants is likely influenced by seasonal variations.

04.02.05 Anthropogenic Tracers to Identify Non-Point Source Contamination and Transport Potential of Septic System Effluent to Surface Waters in Rural Hamlets, Ontario, Canada

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Septic systems are commonly used to dispose of domestic wastewater in rural areas where access to a municipal treatment system is not practical. Improved monitoring of contaminant discharge from septic systems in a range of geological settings is essential to fully understand the impacts on the surrounding environment. Surface water samples were collected up to twice monthly over one year from upstream and downstream of three rural hamlets in Southern Ontario, Canada that exclusively rely on septic systems for disposal of domestic wastewater. A suite of anthropogenic tracers, both biological and chemical, were used to distinguish domestic wastewater contamination from agricultural sources in the investigated hamlets. The tracers included an artificial sweetener (acesulfame-K), pharmaceutical compounds (caffeine, carbamazepine, gemfibrozil, ibuprofen, naproxen, and sulfamethoxazole), and the host-specific fecal indicator bacteria, *Bacteroides* (BacHum and BacBov). Results show that higher concentrations of anthropogenic tracers (Spearman Rank Order Sum test, $P < 0.05$) were consistently observed downstream of the investigated hamlets, indicating the presence of non-point source contamination from septic systems. The tracers with the most elevated concentrations across all three sites were acesulfame-K and BacHum. Elevated concentrations of phosphorus (total and dissolved) and ammonium observed downstream of two sites suggest that nutrients are likely derived from septic systems in these densely populated rural areas. The use of a combined-tracer approach, involving a conservative tracer acesulfame-K coupled with the human specific biological indicator *Bacteroides* (BacHum), helps to determine sources of contamination and also allows for a greater understanding of the potential transport of septic system effluent to nearby streams.

04.02.06 Artificial Sweeteners Reveal Groundwater Contaminated by Historic Landfill Leachate Versus Modern Wastewater

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Historical municipal landfills (~closed >3 decades), typically lacking engineered liners and leachate collection systems, are frequently located by urban streams. The discharge of leachate contaminated groundwater from these landfills poses a threat to stream aquatic ecosystems, especially to endobenthic organisms. Field investigations of this threat can be complicated by influences from groundwater contaminated by modern wastewater, such as from leaky sewer systems, a common occurrence in urban areas. In this study, we demonstrate how a set of four artificial sweeteners (AS): saccharin (SAC), cyclamate (CYC), acesulfame (ACE), and sucralose (SUC), can distinguish groundwater contaminated by these two sources discharging to an urban stream in Ontario, Canada, in comparison to traditional indicator parameters. Groundwater discharge areas influenced by a historic landfill from the 1960s had elevated SAC, and sometimes CYC, and low concentrations of ACE and SUC (both commercialized in the 1990s). Conversely, areas affected by modern wastewater were identified by high ACE and SUC concentrations. The AS were more robust than traditional indicator parameters (e.g., ammonium, major ions) because many traditional indicators are elevated in both leachate and wastewater and are therefore unable to effectively distinguish these two sources when both are present. Similarly, contaminants of emerging concern such as per- and polyfluoroalkyl substances (PFAS) can be found in both leachate and wastewater. At this field site, elevated PFAS concentrations (max. of 31 µg/L for Σ_{27}PFAS) were detected in discharging groundwater, with the primary source determined to be the landfill based on the patterns in AS. These findings are relevant for effective monitoring and assessment of historic landfill contaminated sites, and more broadly, for understanding groundwater contaminant exposure to stream benthic ecosystems at urban stream sites.

04.02.07 Evaluating Seasonal Release of Paraben Transformation Products Formed During Wastewater Treatment

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This study focuses on parabens, a class of compounds used as preservatives and their transformation products resulting from wastewater treatment. This research evaluates seasonal differences in both parent and transformation product release from two wastewater facilities that use different disinfection processes. The first site uses chlorination, and the second site uses UV disinfection. Composite samples were taken for influent and effluent at both sites during the winter, spring, and summer. Sampling events consisted of eight different days with three sampling events per season. Analytes were extracted and quantified using validated extraction and analysis methodologies. Four common parabens and nine transformation products were quantified in influent and effluent at both sites in order to evaluate transformations during treatment as well as release into surface water. The four 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. Winter samples have been analyzed . Chlorinated parabens were detected in influents at both treatment plants, likely due to the use of chloramine used in drinking water treatment upstream. PHBA was the compound quantified in greatest concentrations in influent at both sites and was detected in every sample. At the site that uses chlorination, greater concentrations of chlorinated parabens were seen in effluent than in influent, meaning that the dichlorination step that occurs before release did not effectively dechlorinate chlorinated paraben transformation products. Dichlorinated species were present in higher concentrations than monochlorinated species. The site that uses UV disinfection had an overall greater transformation rate, resulting in a larger percent decrease in parent paraben concentration. Currently spring samples are being evaluated and summer samples will start being collected in June. The different seasons will be compared to determine if released transformation product concentrations vary by season.

04.02.08 Effects of Stormwater Infiltration on Contaminants in Treated Wastewater

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Increasing water scarcity and aging wastewater infrastructure are two major challenges US is facing. Water scarcity affects drinking water availability, wildlife habitats, and also industrial and irrigation water supplies. Wastewater reuse is a commonly proposed strategy to combat water scarcity, and there is ongoing work to develop advanced water treatment methods to enable potable water reuse. However, these technologies are unlikely to be widely implemented in the near future. Most wastewater treatment plants in the US only provide secondary treatment, and many facilities treat a large amount of stormwater in addition to municipal wastewater. Some regions purposely combine storm and sanitary sewers, while others have aging separate systems that allow for unintentional mixing of stormwater and municipal wastewater. Stormwater infiltration causes high variability in the quality and quantity of both raw wastewater and wastewater effluent, which affects the suitability of reclaimed water for potable and non-potable applications. In this project, we collected effluent samples from two wastewater treatment plants in the eastern US: one that serves an area with combined sewers, and another with an aging separate sewer system where significant leakage occurs. We compared contaminant fingerprints in samples from wet and dry weather at the two locations. We quantified a range of organic and inorganic contaminants (e.g. antibiotics, UV-filters, heavy metals) and used non-targeted analysis to investigate the broader range of organic chemicals present in the samples. Our results work towards building an understanding of the variation in wastewater effluent quality, and towards developing safe plans for wastewater reuse using existing infrastructure.

04.02.09 A National Program to Monitor Chemical Substances in Canadian Municipal Wastewater

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A national wastewater monitoring program was initiated by Environment and Climate Change Canada (ECCC) in 2008. Its aim is to assess concentrations of chemical substances in wastewater and biosolids in order to evaluate their importance as exposure pathways in support of risk assessment and risk management of those substances. To date, we have collected samples from over 80 WWTPs across Canada representing typical wastewater treatment processes: facultative and aerated lagoons, enhanced primary treatment, secondary aerobic biological treatment, and advanced biological nutrient removal. Sludge treatment types evaluated include dewatering only, aerobic and anaerobic digestion, pelletization, and alkaline treatment. This program has generated data sets on over 20 substance groups including polybrominated diphenyl ethers (PBDEs) and other halogenated and organophosphate flame retardants, per- and polyfluorinated alkyl substances (PFAS), bisphenol A, metals, triclosan and other pharmaceuticals and personal care products (PPCPs), and hormones. Wastewater and sludge treatment processes are not designed to remove contaminants. However our results demonstrate that some contaminants are removed from wastewater by degradation, volatilization, and/or partitioning to solids, while others are recalcitrant and remain in effluents and final stabilized biosolids. Biological treatment processes tend to show better removals than physical-chemical processes in wastewater treatment systems. In sludge treatment, some substances accumulate during anaerobic digestion, some appear to be degraded during aerobic digestion, and alkaline treatment can cause a dilution effect. However, the removal efficiency of contaminants through both wastewater and sludge treatment systems vary widely across the treatment plants and chemicals studied. Interestingly, the concentrations of some contaminants such as some PFAS, were higher in effluent compared to influent and in final stabilized biosolids compared to raw sludge at some WWTPs. For PFAS, this formation likely occurred due to transformation of unmeasured precursors during wastewater and biosolids treatment. Data generated from this program are publicly available online (https://open.canada.ca/). With collaborators, new emerging work includes assessment of plastics and chemical mixtures, surveillance of COVID-19 residues, and antimicrobial resistance.

04.02.10 PFAS in Landfill Leachate: Extent and Patterns From Recent Studies

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Landfills store large amounts of waste containing per- and polyfluoroalkyl substances (PFAS) from multiple sources including domestic, industrial, wastewater treatment, hazardous waste treatment and more. Given their persistence, PFAS present a unique waste treatment challenge with the conversion of PFAS into persistent perfluoroalkyl acids through wastewater treatment and the return of PFAS as biosolids back into the landfill. Older unlined landfills and leaks from landfills also present a ground and surface water contamination risk. Another emerging issue is emissions of volatile PFAS from landfill gas. Leachate analysis for PFAS can be challenging due to matrix complexity, interferences and widely varying PFAS levels by landfill type. In this study, we summarize recent projects we have conducted on PFAS in landfill leachate. We validated LC-MS/MS methods for 40 PFAS including perfluoroalkyl carboxylates (C_4-C_{14}), sulfonates (C_4-C_{10}, C_{12}), fluorotelomer carboxylates (3:3, 5:3 and 7:3) and sulfonates (4:2, 6:2 and 8:2), C_8 sulfonamides (FOSA, MeFOSA, MeFOSAA, MeFOSE, EtFOSA, EtFOSAA, EtFOSE) and ether carboxylates (HFPO-DA, ADONA, NFDHA, PFMBA and PFMPA) and sulfonates (F-53B and PFEESA). We used PFAS best practices including isotope dilution quantification, pre-screening, weak anion exchange cleanup and multiple mass spectrometric transition monitoring. We analyzed landfill leachates from multiple landfill types including municipal, incinerator waste and hazardous waste landfills from watersheds across North America. Preliminary results indicate that concentrations of PFAS from landfills vary by many orders of magnitude depending on waste input type, with hazardous waste landfills showing the highest concentrations, as expected due to historical disposal of AFFF-containing waste. PFAS incidence and patterns also varied by landfill type with municipal-waste landfills showing characteristic patterns of fluorotelomer carboxylic acids.

04.02.11 Influence of Conjugation on the Fate of Pharmaceuticals and Hormones in Canadian Wastewater Treatment Plants

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Municipal wastewater effluent discharges and land application of treated biosolids are important pathways of chemical substances, including pharmaceuticals and hormones, to the environment. Some pharmaceuticals and hormones are conjugated in the human body through attachment of a sulfate or glucuronide functional group in order to increase water solubility and excretion and these substances subsequently arrive at wastewater treatment plants (WWTPs) in both their conjugated and native (original) forms. Conjugation can be reversed by naturally-occurring enzymes found in WWTPs, which could lead to higher concentrations of free pharmaceuticals/hormones in effluent compared to influent. The objective of this study was to assess the influence of conjugation and deconjugation on the fate of 29 pharmaceuticals (including select antidepressants, antibiotics, anti-epilepsy, and artificial sweeteners) and 17 hormones in wastewater and biosolids from eight and 15 WWTPs, respectively. To measure the impact of deconjugation, deconjugating enzymes (β-glucuronidase/sulfatase) were applied to an aliquot of each sample in order to release target analytes from their glucuronate and sulfate forms. Surprisingly, our results showed that deconjugation during wastewater treatment influenced the fate of only one of the analytes evaluated, lamotrigine, which is an anti-epileptic medication. The removal of lamotrigine was underestimated unless its conjugated form was considered in lagoons and secondary and advanced treatment facilities but not in the primary treatment plant, likely due to the absence of biological activity. With respect to the other pharmaceuticals evaluated, wastewater and biosolids concentrations in the original samples were comparable to the deconjugated ones, indicating that deconjugation was not an important process influencing their fate in WWTPs. Surprisingly, our data indicated that the conjugated form of the six consistently detected hormones (i.e., androstenedione, androsterone, estrone, mestranol, progesterone, and testosterone) were surviving wastewater and biosolids treatment in the WWTPs. The hormones that exit WWTPs still in conjugated form could eventually become deconjugated and hence more problematic in the natural environment. Therefore, evaluation of the original samples only could potentially underestimate the concentration of hormones entering the environment via WWTPs.

04.02.12 Characterization of Specific Wastewater Sources in the South Florida Environment: Deering Estate Rehydration Project Case Study

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Natural and anthropogenic sources contribute a unique signature of elements and chemicals into water creating a "water fingerprint" by assessing water chemistry parameters (conductivity, pH, DO, turbidity, temperature), optical properties (fluorescent dissolved organic matter, colored dissolved organic matter, aromaticity indexes, SUVA Index, chlorophyll, 3D EEMs), elemental composition (nutrients, hydrogeochemical tracers, tracers, and metals), and organic wastewater tracers by the use of High-resolution mass spectrometry (HRMS). This project has generated a database of a combination of physical and chemical water properties by developing and applying methods for the detection and quantification of natural and anthropogenic chemicals in water samples from locations in South Florida's Deering Estate Flow-way. Deering Estate is included in a coastal wetland rehydration project implemented under the Comprehensive Everglades Restoration Plan (CERP). Natural and anthropogenic components have been traced moving through interconnected systems that is comprised of freshwater environments (Everglades-based), Urban impacted areas (septic tank driven) and Coastal areas (Biscayne Bay) endmembers. Sucralose (3687-64 ng/L) has been traced throughout the system significantly declining as the water travels from the septic driven groundwater, to the canals, through Deering Estate, and finally ending in Biscayne Bay providing a conservative tracer of wastewater in the area. Significant differences were found in the composition of groundwater (caffeine, carbamazepine, estriol, sucralose, TP, and TN) pointing out a complex circulation pattern. Endocrine disrupting compounds (EDCs) and phosphate flame retardants (PFR) have been assessed throughout this project for their potential usage as unique wastewater tracers. Other trends in surface waters have been identified with compounds such as carbamazepine(16.5-ND ng/L), caffeine (28.0-0.8 ng/L) and nutrients like total phosphorus (0.0262-0.0055mg/L). Most of these trends could be attributed to dilution from the urbanized areas. Waters from different locations and their temporal variability have been documented and unique parameters so specific to a source that the water can be traced in a conservative manner have been potentially identified.

04.02.13 Chlorinated Paraffins (CPs): A Simplified Analytical Method for Analysis of CPs in Water, Solids, Biosloids and Tissue

M. Woudneh, SGS AXYS Analytical Services Ltd.; B. Chandramouli, SGS AXYS; C. Hamilton, SGS AXYS Analytical Services Ltd. / Client Services; R. Grace, SGS AXYS Analytical Services Ltd.

Chlorinated paraffin (CPs) are high-volume production chemicals with widespread applications as lubricants, flame retardants, and plasticizers. However, shortages of standardized analytical methods have hampered environmental monitoring for these important contaminants. In this project, we have developed an analytical method suitable for monitoring short-chain (C10 –C13, SCCPs), medium-chain (C14–C17, MCCPs), and long-chain (C18-20, LCCPs) chlorinated paraffins. The method is based on liquid-liquid or liquid-solid extraction with dichloromethane followed by chromatographic cleanup using layered acid/base silica and alumina columns. Analysis was conducted using ultrahigh performance liquid chromatography-tandem mass spectrometry. The developed method was validated by replicate (n=5) spike/recovery experiments in water, solid (soil and sediment), and salmon tissue. The method's accuracy/recovery ranged 88-114% in water, 90-119% in solids, and 89-127% in tissue. The method precision/RSD values were comparable between the various matrices and ranged from 2-12%. The developed method was further validated by analyzing NIST 2585 solid, as well as, NIST SRM 1974c mussel, and NIST SRM 1945 whale blubber. The results were compared with previously published values for these standard reference materials. The method reporting limits ranged from 1-5 ng/g for SCCPs, 0.5 to 10ng/g for MCCP, and 2 -65 ng/g for LCCPs using a 10 g sample. The utility of the method was further demonstrated by the analysis of effluent, sediment, and biosolid samples. The method presented in this study can be used to support environmental monitoring of CPs in the aquatic, terrestrial and biotic environments, which can ultimately be used to mitigate risks posed by CPs to the environment.

04.02.14 Trace Detection of Synthetic Cannabinoids in Wastewater

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Synthetic cannabinoids are agonists of the cannabinoid receptors and impact physiological and cognitive processes for humans. Analysis of excreted cannabinoids in wastewater provide relevant information of a defined residential area and the community consumption of these compounds. Recovery of synthetic cannabinoids resultant of complex wastewater matrices is difficult due to hydrophobic properties and chemical fate. An optimized liquid chromatography tandem mass spectrometry (LC-MS/MS) has been utilized for the recovery of potential synthetic and phyto-cannabinoids and respective metabolites from local wastewater treatment facilities.Method validation are to include linearity, selectivity, recovery, ion-suppression, filtration losses and matrix effects. Solid phase extraction (SPE) are to be compared, with comparable limits of quantification between 0.001 and 0.5 µg/L in wastewater. Filtration of samples have previously been found to reduce the recovery for a majority of the investigated cannabinoids. The extraction method is to be applied to 8 different catchment areas (Rush Creek and Trinity Lake) across West Texas to gauge the community use of the cannabinoids in the study. The cannabis biomarker THC-COOH was quantified at all locations, and cannabidiol is to be measured at all eight catchment areas. Quantitative analysis of relative concentrations and recovery loss are to be reported. Findings of the study are to be reported to provide insight on community usage of synthetic cannabinoids in West Texas. Results are to be shared.

04.02.15 Analysis of Monitoring Data for the Fragrance Material HHCB in Surface Waters of the United States From 2015-2021

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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 has been in use since the 1960s in a variety of consumer and commercial products. Such synthetic musks have attracted attention for several decades because of their prolonged presence in environmental samples and found in human mother's milk (HERA 2004). In 2014, the U.S. Environmental Protection Agency (EPA) completed a final risk assessment that indicated no concern for the use of HHCB as a fragrance ingredient in commercial and consumer products in the U.S. In December 2019, EPA finalized the designation of chemical substances as a high-priority for risk evaluation under the Toxic Substances Control Act (TSCA), including HHCB; TSCA was amended in June 2016 giving EPA broader authority to conduct risk evaluations for conditions of use of high priority chemicals and HHCB was included despite the 2014 EPA finding. Monitoring data for HHCB in freshwater sources in the United States collected since the EPA risk assessment (2015 to 2021) were collected from the United States Geological Survey National Water Information System database. Of the 1,019 surface water samples identified, 690 were below the limit of detection (0.04 – 0.16 µg/L) and only two samples exceeded 1 µg/L. These observations were consistent with the previous 2014 EPA analysis, however, the detection limit for HHCB in freshwater declined by roughly an order of magnitude between the 2000's and 2010's. Moreover, current use of HHCB in the United States does not appear to present a significant risk to organisms in the aquatic freshwater environment as several recent environmental risk assessments of HHCB have proposed predicted no-effect concentrations greater than 1 µg/L.

04.02.16 Trace Organic Contaminant (TOrC) Discovery and Monitoring in Onsite Wastewater Treatment Systems Using High-Resolution Mass Spectrometry

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Onsite septic systems introduce a wide range of trace organic contaminants (TOrCs) to the environment through effluent discharge resulting in contaminated soil, groundwater and receiving surface waters. Nitrogen Removing Biofilters (NRBs) are innovative/alternative onsite wastewater treatment systems that use established microbial communities and natural materials to treat septic tank effluent. Based on previous work, it is known that 24 targeted pharmaceuticals and personal care products (PPCPs) are removed during NRB treatment via chemical and biological removal mechanisms. It has also been found that NRB performance recovers after a realistic antibiotic spike. While NRBs are being optimized for nitrogen removal, there remains much to learn regarding their performance in removing organic contaminants. Furthermore, the potential impacts of TOrCs with antimicrobial action on NRB performance have not been extensively studied. In this work, a suspect screening approach is applied to identify and prioritize TOrCs of potential concern in domestic wastewater. Septic tank and NRB effluent samples were collected from seven previously installed NRB systems across Suffolk County, NY and analyzed using liquid chromatography and quadrupole time of flight mass spectrometry (LC-QTOF-MS) to screen for TOrCs. Detections have been confirmed for twenty targeted PPCP analytes frequently detected in septic tank effluent using an established positive ion method on an Agilent 6545 QTOF mass spectrometer. Here we will present initial findings on detection frequency, spatiotemporal variability, and abundance of TOrCs in household wastewater and discuss how this data may inform future NRB design.

04.02.17 An Inherent Biodegradation Test Using a Modified OECD 301B Ready Biodegradation Test Design, Presenting Additional Extended Test Results

S.P. McLaughlin, Smithers - Environmental Risk Sciences / FATE

The OECD Ready Biodegradation and the Inherent Biodegradation Test Methods have provided years of guidance for assessing the persistence of organic chemicals. If a test substance exposed to a dilute inoculum generally comprised of activated sludge achieves 60% biodegradation equivalent to 60% of its organic carbon being converted to CO_2 within a 10-day window (once reaching 10% CO_2 evolution) during the 28-day test, the test substance can be labeled 'readily biodegradable'. If the test substance does not pass this criteria, it can then still be tested for persistence using Inherent Biodegradability Tests described in the OECD 302 Series. However, for chemicals that are insoluble, many of the current 302 test methods provide limited testing options when trying to evaluate mineralization of the test chemical to CO_2. In this experiment, an OECD 301B test design was conducted with one of the standard reference substances, sodium benzoate, but at increasing amounts of the activated sludge inoculum. This was conducted to determine how much inoculum can be used in a test system designed to monitor CO_2 evolution from the biodegradation of the test chemical while keeping endogenous CO_2 from the blank inoculum to a minimum in order to calculate the percent biodegradation of the test chemical. The concentrations used in this experiment were 100, 200 and 300 mg/L as dry sludge solids. The results will demonstrate if the method is appropriate at concentrations of sludge up to 3 to 10 times higher than what is allowed in the Ready Biodegradation Tests, but on the lower range than what is typically used in the OECD 302B tests, which only measure DOC decline and are not appropriate for water insoluble compounds. During this experiment it was shown that the 100 mg/L dry sludge solid inoculum was the most appropriate. Additional test compounds such as cellulose and metformin were selected to implement the method and new data beyond day 28 is presented to show improved biodegradation in the solutions with increased inoculum versus the standard OECD 301B test solutions.

04.02.18 Adsorption of Copper, Cadmium, 9,10-Dimethylatedanthracene and Pentabromoethylbenzene on Canadian Quick Clays

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Industrial activities and the use of pharmaceutical products cause the presence in the environment of a growing number of pollutants having various physicochemical properties. Some emerging contaminants, like novel flame retardant, or heavy metals escape water treatment processes and are found in drinking water. Natural materials, like clays, are an efficient way for the removal of different physico-chemical proprieties pollutants. Canadian Quick clays (QC) are uplifted glaciomarine natural clays from the past glaciation located mostly in the northern coastal region like the St-Lawrenceecosystem (SLE). To the best of our knowledge, no work has investigated yet the capacity of Canada Eastern QC to remove and trap pollutants from water. The aim of our work is to study the sorption, desorption and sequestration of various pollutants such as heavy metals (cadmium, copper) di-methylated polycyclic aromatic hydrocarbon (9,10-dimethylatedanthracene), and a flame retardant (pentabromoethylbenzene) on QC. QC capacities to adsorb and trap pollutantswere evaluated using kinetics and sorption-desorption isotherms. In addition, our study investigates the effects of temperature and salinity during the sorption process. Clay's characterization was performed by X-ray diffraction (XRD), X-ray fluorescence (XFR). Montmorillonite, having high adsorption capacity, was used as reference. Isotherms experiments were fitted with both Langmuir and Freundlich models. Results showed that QC are having high absorption capacities for copper and cadmium. Average copper maximum sorption capacities (q_m) are respectively 4.44 and 3.04 mg/g for quick clays and Montmorillonite. Kinetics pseudo-second order constants (K_2) were also determined for these materials (quick clays: 5.16 - 17.4 µg $g^{-1}min^{-1}$; Montmorillonite: 0.77 µg $g^{-1}min^{-1}$). Higher copper distribution constants (K_d) were estimated for quick clays (34.8 at 1500 µg/L and 2.52 at 15000 µg/L) than Montmorillonite (24.9 at 1 500 µg/L and 0.51 at 15 000 µg/L) at initial concentrations of 1500 and 15000 ug/L. Sorption processes on quick clays were found to be spontaneous and showing high adsorption at low metal level concentrations and showed exothermic and endothermic behaviors at lower and higher equilibrium concentration, respectively.

04.03 Elucidating Chemical Transformations in Environmental Fate Research

04.03.01 Transformation Product Formation Upon Heterogeneous Ozonation of the Tire Rubber Anti-Oxidant N-1,3-Dimethylbutyl-N-Phenyl-P-Phenylenediamine

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Transformation products (TPs) are a class of widespread emerging contaminants whose environmental fate and risks are poorly understood. We investigated TP formation occurring upon heterogeneous solid-gas-phase ozonation of the common tire rubber antioxidant 6PPD (N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine). Oxidative transformation of 6PPD occurred during exposure to ozone, and various TPs were examined, especially the 6PPD-Quinone, a chemical reported to posing acute toxicity on pre-spawn coho salmons. Aside from pure 6PPD, the tire tread wear particles (TWP) ozonation were also investigated and multiple TPs were found to be generated during the ozonation process. By screening environmental samples, we prioritized a few TPs that formed from 6PPD ozonation (in both pure compound and TWP) as environmentally relevant in receiving waters impacted by roadway runoff. The data imply that tire rubber antioxidants react with ozone to form various TPs that are released throughout the tire rubber service life and have potential impacts on aquatic environments.

04.03.02 High Abundance of Antioxidants and Their Transformation Products in Traffic-Influenced Urban Air from a Retrospective Analysis of Passive Air Samplers

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Antioxidants are chemicals mixed with functional materials such as lubricant oil and rubber to minimize damage caused by oxidants in the environment. This is achieved because the antioxidant is in large quantities embedded in the material are more reactive with environmental oxidants such as OH radicals and ozone than the material itself. While different diphenylamine related antioxidants have been detected in wastewater and natural surface water, the environmental occurrence and risk of their transformation products are less-known until recently. Tian et al. (2021) comprehensively characterized the oxidation product (6PPD-quinone) of N-(1,3-dimethylbutyl)-N'-phenyl-1,4-benzenediamine (6PPD) that is widely used in rubber. While road runoff is found as one pathway for 6PPD-quinone to enter the aquatic environment and cause ecotoxicity, emissions of 6PPD and various other antioxidants to air, and their atmospheric transformations and depositions remain uncharacterized. The overall objective of this study is to characterize the atmospheric pathway linking the chemical sources and receptors in the environment and assess the overall risk including both parent compounds and the transformation products. As the first step, we conducted a retrospective analysis on passive air samplers deployed at urban sites having different degrees of influence from road traffic. The extracts of the passive air samplers were analyzed using a GC-TOFMS. Based on accurate mass determination, isotope patterns and model predicted retention indices, suspect peaks of 6PPD and 6PPD-quinone were found in the urban air samples. Their abundances in the samples at a road traffic-influenced site were one order of magnitude higher than that at a residential site. In addition to 6PPD and 6PPD-quinone, we also found suspect peaks of tertoctyl-diphenylamine and its oxidized species, both of which were over two orders of magnitude higher in the samples than in the field blanks. However, unlike 6PPD, the substituted diphenylamine and its oxidized species occurred at similar levels at road traffic influenced sites and the residential site. This retrospective analysis has led to our further monitoring and modelling investigations on the emissions, atmospheric transport, transformation and deposition of antioxidants as a complex mixture in urban environments, including the global context through the GAPS Megacities project.

04.03.03 Biotransformation of 2,4,6-tris(2,4,6-tribromophenoxy)-1,3,5-Triazine (TTBP-TAZ) Can Contribute to High Levels of 2,4,6-Tribromophenol (2,4,6-TBP) in Humans

G. Zheng, Indiana University / O'Neill School of Public and Environmental Affairs; L. Melo, R. Chakraborty, Indiana University; J. Klaunig, Indiana Unversity; A. Salamova, Indiana University / SPEA

2,4,6-tribromophenol (2,4,6-TBP) is the most widely produced brominated phenol that accumulates in human tissues and is a potential toxicant. Previous biomonitoring studies found 2,4,6-tribromophenol (2,4,6-TBP) levels in human tissues were significantly higher than those for polybrominated flame retardants measured in the same samples. However, the direct intakes of 2,4,6-TBP via dust ingestion, air inhalation, dermal contact with consumer products and dietary were minimal. Hence, the identification of indirect exposure sources to 2,4,6-TBP via biotransformation in humans has been made in this study. First, *in vitro* assays utilizing human and rat liver microsomes were applied in this study to compare the biotransformation rates of four brominated flame retardants, including 2,4,6-tris-(2,4,6-tribromophenoxy)-1,3,5-triazine (TTBP-TAZ), decabromodiphenyl ethane (DBDPE), 1,2-bis(2,4,6-tribromophenoxy) ethane (BTBPE) and tetrabromobisphenol A (TBBPA). Our results showed that TTBP-TAZ was rapidly metabolized in both human and rat liver microsomes with a half-life of 1.1 and 2.2 h, respectively, suggesting TTBP-TAZ was the potential candidate of 2,4,6-TBP precursor in biota. Second, suspect and target screening strategies were applied to explore the metabolic pathway of TTBP-TAZ in which 87% of TTBP-TAZ was able to generate 2,4,6-TBP in human liver microsomes. Third, the *in vitro* results were further tested by an *in vivo* experiment in which 2,4,6-TBP was detected in the rat blood and liver at concentrations of 50 ± 14 and 270 ± 110 µg/g lipid weight, with the average transformation rate (TTBP-TAZ to 2,4,6-TBP) of 1.3% and 0.6%, respectively, after being exposed to 250 mg/kg body weight/day TTBP-TAZ for a week. The hepatic mRNA expression levels of rats revealed that the strongest toxicity of TTBP-TAZ was the activation of aryl hydrocarbon receptor (AhR) receptors and the promotion of fatty degeneration (18 and 28 fold change compared to control, respectively). Considering the widespread occurrence of TTBP-TAZ in our environment, its significant contribution to the formation of 2,4,6-TBP in humans and the strong endocrine disrupting toxicity of 2,4,6-TBP, our findings warrant further toxicological research focused on TTBP-TAZ.

04.03.04 Aqueous Ozonation of Furans: Kinetics and Transformation Mechanisms Leading to the Formation of α,β-Unsaturated Dicarbonyl Compounds

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Despite the widespread occurrence of furan moieties in synthetic and natural compounds, their fate in aqueous ozonation has not been investigated in detail. Reaction rate constants of seven commonly used furans with ozone were measured by competition kinetics, which is known to be dependent on the type and position of furan ring substituents. Transformation product analysis of the reaction of furans with ozone focusing on the formation of toxic organic electrophiles using a novel amino acid reactivity assay revealed the formation of α, β-unsaturated dicarbonyl compounds, 2-butene-1,4-dial (BDA) and its substituted analogues (BDA-Rs). Their formation could be attributed to ozone attack at the reactive α-C position leading to furan ring opening. The molar yields of α, β-unsaturated dicarbonyl compounds varied with the applied ozone concentration for different furans. The identified α, β-unsaturated dicarbonyls are well-known toxicophores that are also formed by enzymatic oxidation of furans in the human body. In addition to investigation into data on kinetics, transformation product analysis and proposed reaction mechanisms for the ozonation of furans, this study raises concern about the presence of α, β-unsaturated dicarbonyl compounds in water treatment and the resulting effects on human and environmental health.

04.03.05 Biotransformation of Aqueous Film Forming Foam Associated Perfluoroalkyl Acid Precursors; an In Vitro and In Vivo Mouse Liver Comparison

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Aqueous film forming foam (AFFF) used to fight liquid fuel fires can contain hundreds of per and polyfluoroalkyl substances (PFASs), including labile precursors and recalcitrant "forever chemicals." Many individuals are exposed to AFFF derived PFASs, either through workplace hazards, as is the case with many firefighters and military personnel, or through drinking water from AFFF contaminated aquifers. Some PFASs are bioaccumulative and have been linked to liver, kidney, and testicular cancers. Previous work analyzing PFASs in mouse serum from AFFF-dosing studies suggests that AFFF-associated precursors can be metabolized *in vivo* to form bioaccumulative perfluoroalkyl acid (PFAA) end products. Here we conducted *in vitro* studies in which C57BL/6 mouse liver S9 assays were incubated with a real AFFF mixture consisting primarily of a historical electrochemically-fluorinated Lightwater formulation. Based on previous work, this Lightwater AFFF formulation contains a wide array of known and novel substituted sulfonamide precursors. Samples were screened for PFASs using a quadruple time of flight – mass spectrometer (QTOF) with negative electrospray ionization, in data independent acquisition mode to scan m/z 100 - 1200. Suspect screening was performed using an extraction ion chromatogram (XIC) list of known and novel AFFF-associated PFASs. Preliminary results suggest formation of perfluoroalkyl sulfonates including perfluorooctane sulfonate (PFOS) during 60-minute incubations. To compare in vitro assays to in vivo processes, liver tissue from C57BL/6 mice dosed with the same AFFF formulation was extracted and analyzed. Preliminary analysis suggests several novel substituted perfluoroalkyl sulfonates, including ketone-PFOS and unsaturated PFOS, accumulated in mouse livers. Here we will compare PFAS profiles from *in vitro* and *in vivo* experiments to investigate biotransformation of AFFF-associated precursors and bioaccumulation of PFASs in liver tissue. This research provides further evidence that precursors contribute to the bioaccumulation of persistent toxic PFASs.

04.03.06 In Silico Molecular Docking Simulations of Methoxylated Polybrominated Diphenoxybenzenes with Human CYP1A1/2 3A4, and 2B6

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The transformation of contaminants can have a significant impact on their environmental fate. Methoxylated polybrominated diphenoxybenzenes (MeO-PB-DiPhOBzs) are novel bioaccumulative contaminants that have been measured in herring gulls (*Larus argentatus*) and their eggs from the Laurentian Great Lakes area. MeO-PB-DiPhOBzs are currently believed to be the products of sequential photolysis and metabolism (e.g. by soil microbiota) of the tetradecabromo-1,4-diphenoxybenzene flame retardant. It is of interest to know if herring gulls are competent in MeO-PB-DiPhOBz demethylation as hydroxy-PB-DiPhOBzs have yet to be measured in any environmental compartment. The objective of the present study was to use *in silico* docking simulations to model the interactions of 20 MeO-PB-DiPhOBz isomers with the active site of cytochrome P450s. Due to the availability of crystal structures from the RCSB Protein Data Bank, templates were built from human CYP1A1/2, 3A4, and 2B6. Docking was performed using the internal coordinate mechanics (ICM) software. Templates prioritized crystal structures that had as high as possible resolution and that had a bound ligand. Ligands were docked into each model using a flexible ligand and rigid protein structure. Isomer binding positions were predicted by a Monte Carlo global optimization procedure, and the ICM virtual ligand screening (VLS) scoring function produced a score to compare binding energies between isomers. Models were evaluated by selecting 20 known binders for each isoform and generating 50 decoy compounds per known binder via the DUD.E database. VLS scores were then normalized for each model based on the scores for binders vs. nonbinders. Mean scoring values for MeO-PB-DiPhOBz docking in each isoform ranked in the order CYP3A4 > CYP2B6 > CYP1A1 > CYP1A2. This is consistent with CYP1A enzymes' affinity for coplanar dioxin-like substrates when compared to CYP3A4 and 2B6. Relative scores for MeO-PB-DiPhOBz isomers were relatively consistent, although were overall greater in tetrabrominated vs. pentabrominated isomers. This may be due to the decreased size and resulting hydrophobic interactions, but may also be a result of the few isomers chosen for simulation. Future work includes performing docking simulations of additional isomers with more variation in the degree of bromination. Additionally, work is ongoing to compare VLS scores to the relative binding affinities determined via *in vitro* liver microsomal stability assays.

04.03.07 Use and Limits of Transformative PFAS Measurement Methods Including TOP and Organic Fluorine

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Many PFAS can transform in the environment into perfluorinated carboxylates/sulfonates (PFCA/PFSA) such as PFOA and PFOS from exposure to sunlight, microbial activity, and through the wastewater treatment process. While the transformation of some PFAS into perfluoroalkyl acids (PFAA) in the environment and through the wastewater treatment process has been studied extensively, these studies have typically involved the measurement of select precursors and their target PFAA products. They do not represent a complete view of all PFAS chemistries within these systems. Recent work using emerging techniques such as non-target analysis has shed more light on the extent and nature of the wide variety of PFAS chemistries potentially present. The limited scope of targeted analysis, and the relative complexity/high cost of non-target analysis preclude its widespread use for understanding total PFAS loads. With limits around the scope of targeted PFAS analysis, and the relative complexity of non-targeted analysis, PFAS measurement techniques that use chemical transformation to estimate total/unknown PFAS can be very valuable. Two of these techniques are the total oxidizable precursor assay (TOP), which uses an oxidative approach to convert precursors into PFCAs prior to targeted measurement, and organic fluorine measurement, which estimates organic fluorine in a sample by measuring the fluoride products from combustion. These measurements are referred to as total but have limits by chemistry and processing prior to instrumentation. In this study, we used TOP assay and organic fluorine to assess the limits of transformation these measurements and evaluate the utility of multiple transformative measurements to assess total or non-target PFAS. The TOP assay procedure was augmented to include tracking of reaction, extraction and evaporative losses using isotopically labeled standards. Other PFAS chemistries, such as per-and polyfluoroether-carboxylate and sulfonate targets were studied in these methods to assess their transformation behaviour in the analysis. Precursor monitoring was also implemented to assure completeness of transformation. Organic fluorine measurements using combustion ion chromatography were performed using published protocols, with limits in terms of the inclusion of PFAS chemistries depending on the extraction or pre-concentration methods. Preliminary results show large increases in PFCAs post-TOP in biosolids. In addition, the ether PFAS showed varying degrees of transformation post-TOP, depending on structure. We will present information on the comparative utility of these transformative techniques in ambient and wastewater monitoring applications

04.03.09 Analysis of the Photodegradation of Benzobicyclon Hydrolysate in the Presence of a Seawater Gradient and Relevant Seawater Ions

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Benzobicyclon (BZB) is a pro-herbicide recently approved for application on rice in California and undergoing research in anticipation of registration in southern states including Louisiana. BZB rapidly hydrolyzes in flooded fields to generate the active ingredient of the herbicide (BUTTE□), benzobicyclon hydrolysate (BH). Previous research has shown BH preferentially partitions into rice field water, while BZB is adsorbed in sediments. BH undergoes photolysis and its half-life varies depending on the characteristics of the water. In distilled water, BH has a reported half-life of 78 hours, 38 hours in rice water, and 3.4 hours in 25 ppt seawater. Previous research by this author has also shown that small amounts of iron, magnesium, and copper are in part responsible for the rapid degradation of BH in seawater when the water isolated from other confounding variables. While there is little risk for the herbicide to be transported from treated systems to non-target ecosystems as a result of spray drift, there is a risk of contamination to ecosystems when flooded fields are drained or in the occurrence of saltwater intrusion. The objective of the current study is to determine if the exacerbating effects of seawater exist when Benzobicyclon is irradiated across a gradient of salinities. This was done to analyze the impact that brackish water may have on the partitioning and degradation of BZB in fields contaminated with seawater. It was found that as salinity increased from 0 ppt to 35 ppt, the half-life of BZB decreased exponentially from approximately 85 hours to 3.5 hours. This study may be able to determine the efficacy of this herbicide under the influences of hurricanes or saltwater intrusion in the future.

04.03.10 Microbial Degradation of Aquatic Herbicides Used for Invasive Plant Control

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Herbicides are commonly applied to lakes to control invasive aquatic weeds throughout the United States. In Wisconsin, several herbicides including fluridone and florpyrauxifen-benzyl (FPB) are registered for use against the invasive Eurasian watermilfoil, a plant that can hinder recreational use of water and outcompete native species. To promote treatment efficacy and responsible use of these herbicides, degradative pathways and environmental fate of each must be well understood. Degradation rates of each herbicide in the environment are variable and are thought to partially controlled by microbial degradation. However, specific microor-organisms responsible for the breakdown of fluridone and FPB have not been identified. This study aims to do this by investigating interactions between these herbicides and microbes in the laboratory and in the field. Specifically, this work includes laboratory-based microcosms, culturing efforts to isolate and identify herbicide-degrading microorganisms in the laboratory, and analysis of the microbial community observed in lakes during and after whole-lake treatments with these herbicides. By studying microbial community shifts in lake water and sediment upon herbicide exposure, measuring herbicide degradation rates in the laboratory, and culturing herbicide-degraders, we take initial steps towards understanding the role microorganisms may play in the degradation of these two herbicides.

04.03.11 Assessing the Impact of Environmental Conditions and Molecular Characteristics on As-Dom Complexation

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Arsenic (As) contamination is a worldwide environmental health issue, and it is crucial to understand mechanisms responsible for As mobility in aqueous systems. Certain chemical interactions, such as complexation with dissolved organic matter (DOM), can reduce the mobility and potential exposure of As by limited the amount of free As in the water column. Changes in environmental conditions (e.g. pH and salinity) can alter both physical and chemical properties of the DOM, thus affecting arsenic-dissolved organic matter (As-DOM) complex formation. This work assesses As-DOM complex formation over a range of pH and salinity treatments in conjunction with different DOM types that comprise of diverse molecular weights and molecular structures. We will use three separate types of DOM: Suwanee Reverse Osmosis Humic acid, DOM derived from two distinct coal samples, and autochtonous DOM from pond water. DOM types will be characterized using high performance liquid chromatography coupled with a size exclusion column (HPLC-SEC) and a fluorescence and absorbance detector. In addition, specific ultraviolet absorbance ($SUVA_{254}$), a proxy for aromaticity, that will be analyzed using an Agilent UV-VIS. As-DOM complexation will be determined through a series of dialysis experiments prepared with various concentrations of sodium metaarsenite (0 – 500 µg/L), pH conditions (3-10), and salinity treatments (0-20 psu) in order to represent a variety of geochemical conditions. Once equilibrium is reached, As concentrations outside and inside the dialysis tube will be analyzed through inductively coupled plasma mass spectrometry (ICP-MS). Results will be used to determine the conditional distribution coefficients, D_{OM}, and to build isotherms based on As concentration, pH and salinity. To investigate the impact of molecular characteristics, we will compare D_{OM} values to molecular structure and molecular weight to establish trends in As-DOM binding. Our results will determine the geochemical conditions with the highest complexation efficiency further contributing to current understanding of As cycling and mobility in aqueous systems.

04.03.12 Comparison of GC/MS + BSTFA Derivatization and Large-Volume Direct Aqueous Analysis Via HPLC/MS/QTOF to Identify and Quantify Phenanthrene Polar Transformation Products

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Phenanthrene (PHE) has been used as an ideal compound for study as it undergoes biotic and abiotic transformation reactions in the environment to form mono-hydroxy, di-hydroxy, and carboxylated transformation products, many of which show greater toxicity than PHE itself. Historically, these compounds were measured using solid-phase extraction and identified by target analysis using an *N,O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA) derivatization method via gas chromatography–mass spectrometry (GC/MS). However, for some polar transformation products, the derivatization GC/MS method resulted in poor recovery efficiencies and did not allow for resolution of some polar and non-target transformation products. A large-volume (900 uL) direct aqueous injection method was developed for the analysis of polar phenanthrene transformation products using high-resolution high-performance liquid chromatography–mass spectrometry quadrupole time-of-flight (HPLC/MS/QTOF). To compare and contrast the GC/MS with derivatization and large-volume HPLC/MS/QTOF methods for the analysis of phenanthrene polar transformation products, we measured these compounds in aliquots from *Mycobacterium Sp.* Strain ELW1 metabolism of phenanthrene, which has been shown to create several mono- and di-hydroxyphenanthrene intermediaries, as well as the primary metabolism end-product of *trans*-9,10-dihydroxy-9,10-dihydrophenanthrene. The advantages and disadvantages of the two methods will be presented.

04.03.13 In Silico Prediction of the Biotransformation of 'Novel' Organophosphate Esters (OPEs): Understanding Metabolic Pathways and Influence on Biological Fate

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Organophosphate esters (OPEs) have seen increased production and use as both flame retardants and plasticizers, often as replacements of the several banned brominated flame retardants (BFRs) such as PBDEs. Several OPEs are ubiquitous in the environment, having been reported worldwide in water, wastewater, sewage, soil, sediment and biota, and often at environmental concentrations higher than the BFRs they replaced. The biotransformation and metabolism of OPEs in biota (e.g. birds, fish and mammal species) has been increasingly studied in recent years, with a focus on a few OPEs such as triphenyl phosphate (TPHP). However, very little is known about the environmental fate, stability and bioaccumulation of 'novel' OPEs due to a lack of toxicokinetic, biotransformation and metabolism data in exposed biota. A growing trend in the industry towards larger and more complex OPE molecular structures adds a new dimension to the biological stability and biotransformation of longer chained, higher molecular weight oligomeric OPEs. To inform the environmental fate and stability of more 'novel' OPEs given these data gaps, the present study predicted biotransformation and metabolism via *in silico* modelling tools, specifically the OECD Toolbox v4.4.1 and EPI Suite^{TM}. OPEs studied included bisphenol-A bis(diphenyl phosphate) (BPADP), tetrakis(2-chloroehtyl)dichloroisopentyldiphosphate (V6), resorcinol bis(diphenylphosphate) (RDP) and tetrakis(2,6-dimethylphenyl)-m-phenylene biphosphate (RDX). Predicted *in vitro* and *in vivo* metabolites in both rat liver S9 and microsomal fractions are presented, with properties predicted for all metabolites such as: sorption, biotransformation rate constants, bioconcentration and bioaccumulation factors (BCF/BAF). Predicted *in*-vivo metabolites of BPADP include bisphenol-A (BPA), phenol, diphenyl phosphate (DPHP), hydroquinone and phenyl phosphate, indicating the involvement of several metabolic pathways in its transformation such as dealkylation, dephosphorylation and hydrolysis. These predictions indicate that larger, complex OPEs are potentially unstable in biota and may lead to a broader array of secondary degradation products. The environmental fate and bioaccumulation of 'novel' OPEs is predicted to be impacted by biotransformation, and future research such as *in vitro* and *in vivo* stability assays should be conducted to determine empirical confirmation.

04.03.14 Using Field and Lab Experiments to Quantify Transformation and Persistence of Aquatic Herbicides

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Herbicides are commonly used in freshwater systems for the control of invasive and nuisance plants. Herbicides are typically applied at low concentrations for long exposure times to effectively treat target plants, but variations in herbicide degradation rates in applications makes appropriate dosing challenging. Additionally, slower than expected degradation rates can result in unintended exposure to non-target organisms. While previous studies have identified single-mechanism degradation pathways in lab and field studies, the fate of aquatic herbicides in the environment is determined by the sum of all biotic and abiotic transformation pathways, physical transport, and partitioning. We used a combination of field and lab studies to identify and quantify the dominant transformation pathways of three commonly used aquatic herbicides: 2,4-dichlorphenoxyacetic acid (2,4-D), fluridone, and florpyrauxifen-benzyl. Laboratory studies were used to quantify microbial degradation in water and water-sediment microcosms, as well as direct and indirect photolysis rates and sorption partition coefficients. Field studies quantified degradation rates of these three herbicides following whole-lake applications by measuring in-lake herbicide concentrations as well as discharge from outlets, sorption to sediments, and partitioning to suspended solids. We measured 2,4-D in lake water up to 70 days after treatment in 5 lakes and found 2,4-D is predominantly degraded by microbes in the lake sediment, with an average half-life of 15 days. In contrast, photodegradation and sorption contributed to the fate of fluridone and florpyrauxifen-benzyl. These results will be used to determine dominant transformation and transport pathways of commonly used aquatic herbicides for improved herbicide applications, while also creating a framework for quantifying environmentally relevant transformations of organic contaminants in complex aquatic systems.

04.03.15 Fate and Effects of Riot Control Chemicals Used in Portland, Oregon, During Summer 2020

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Widespread protests for racial justice during the summer of 2020 evolved into confrontations with police in several US cities, most notably Portland, Oregon. The police response in Portland included intensive and extended use of riot control agents (RCAs) in an urban environment, which led to concern over the environmental fate and effects of the RCA constituents and their breakdown products. There are no prior studies that directly address these issues under comparable scenarios, and monitoring of RCA-derived contaminants in the affected area was limited. Under such circumstances, any assessment of the risks to environmental and human health requires substantial use of models that can predict the properties, fate, and risk of chemicals in the environment. To demonstrate the power and limitations of this approach, we will present the use of RCAs in Portland as a case study in "ab initio" (i.e., before measured data is available) prediction of the environmental fate and risks of chemical contaminants. For organic chemicals such as the active ingredients in tear gas (2-Chlorobenzalmalononitrile, CS), well-established predictive models are available for the key environmental fate determining properties such as Henry's law constant and octanol-water partition coefficient. However, predicting the rates and products of transformation of these chemicals (e.g., by hydrolysis or photolysis) requires more-recently developed methods that combine rule-based expert systems, machine learning, and computational chemistry. While these models are readily applied to lachrymators like CS, there is little directly-relevant data to use for validation. Other RCAs include metals (e.g., Zn and Al in "HC smoke"), and most are deployed in products that contain pyrotechnics based on metals and inorganic propellants like perchlorate. The deployment of RCAs with pyrotechnics also means that their residues will be mixtures of active ingredients and combustion products. Predicting the environmental and/or health effects of these mixtures is currently beyond the scope of any available models.

04.03.16 Quantification of Environmental Fate of Dinitroanisole and Nitrotriazolone in Freshwater Sediment Systems Using Stable Isotope Tracers

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Environmental release of Insensitive high explosive (IHE) compounds dinitroanisole (DNAN) and nitrotriazolone (NTO) is of great concern due to their high polarity and associated higher potential for offsite migration in surface water environments. The goal of this study is to evaluate and quantify adsorption and mineralization of these compounds in freshwater sediment systems using environmentally relevant concentrations. We conducted aquaria-scale experiments using three geochemically and texturally contrasting freshwater sediment types: low organic carbon (OC) river sand, high OC pond silt and high OC wetland sediment. 50 L aquaria containing sediment were equilibrated over three weeks prior to compound addition, maintaining oxic water column and hypoxic/anoxic sediment layer. Isotopically dual labelled (^{13}C and ^{15}N) DNAN and NTO were introduced into separate aquaria 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. Water samples were analyzed for munitions (parent compounds and organic derivatives) and compound-derived mineralization products including inorganic nitrogen species ($NO_{2,3}^-$, NH_4^+, N_2O, and N_2) and dissolved inorganic carbon (DIC: $CO_{2aq} + H_2CO_3 + HCO_3^- + CO_3^{2-}$). Sorbed fractions of parent compounds, organic derivatives and compound-derived NH_4^+ were analyzed in sediment samples. DNAN was removed from the water column at a higher rate than NTO in all sediment types. DNAN and its organic derivatives considerably sorbed onto the sediment while NTO did not show measurable sorption in sediment. Sediment sorption of DNAN and derivatives will be quantified and compared among different sediment types. Smaller grain size coupled with higher OC content in sediment is more important for NTO mineralization. Formation of distinct mineralization products will be quantified separately, and total mineralization rates of these compounds will also be computed via summing all measured mineralization products. Comprehensive evaluation of quantified mineralization will propose mineralization pathways and identify possible factors affecting mineralization. Resulting comparative estimates of mineralization and adsorption kinetics of DNAN and NTO from our study have the potential to aid in parameterization of fate and transport models and contaminant management schemes.

04.04 Human Exposure to Organic Contaminants of Concern

04.04.01 Quaternary Ammonium Compounds: Bioaccumulation Potentials in Humans and Levels in Blood Before and During the COVID-19 Pandemic

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Quaternary ammonium compounds (QACs) are commonly used in a variety of consumer, pharmaceutical, and medical products. In this study, bioaccumulation potentials of 18 QACs with alkyl chain lengths of C8-C18 were determined in the in vitro–in vivo extrapolation (IVIVE) model using the results of human hepatic metabolism and serum protein binding experiments. The slowest in vivo clearance rates were estimated for C12-QACs, suggesting that these compounds may preferentially build up in blood. The bioaccumulation of QACs was further confirmed by the analysis of human blood (sera) samples (n = 222). Fifteen out of the 18 targeted QACs were detected in blood with the ΣQAC concentrations reaching up to 68.6 ng/mL. The blood samples were collected during two distinct time periods coinciding with the outbreak of the COVID-19 pandemic (n =111) and earlier (n = 111). The ΣQAC concentrations were significantly higher in samples collected during the pandemic (median 6.04 ng/mL) than those measured in samples collected before the pandemic (median 3.41 ng/mL). This is the first comprehensive study on the bioaccumulation potentials and biomonitoring of the three major QAC groups and our results provide valuable information for future epidemiological, toxicological, and risk assessment studies targeting these chemicals.

04.04.02 Characterization of Polyfluorinated Alkyl Substances (PFASs) in Commercial Anti-Fog Products and Their In Vitro Adipogenic Activity

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Due to the COVID-19 pandemic, there has been an increased demand for anti-fog products for use on eyeglasses while wearing face masks. This study sought to characterize PFAS compounds in 4 commercial anti-fog sprays and 5 cloth products. Fluorotelomer alcohols (FTOHs) and fluorotelomer ethoxylates (FTEOs) were detected in every product. 6:2 FTOH and the 6:2 FTEO polymeric series were the predominant PFAS compounds observed. 6:2 FTOH ranged from 3.19 ± 0.09 to 11,000 ± 704 µg/mL for anti-fog sprays and 10.9 ± 1.73 to 373 ± 18.0 µg (g cloth)$^{-1}$ in anti-fog cloths. While 6:2 FTOH and the 6:2 FTEO polymeric series were predominant in products, one anti-fog cloth (Cloth A) contained 8:2, 10:2, 12:2, 14:2, and 16:2 FTOH and FTEO polymeric series. Both 8:2 FTOH and 10:2 FTOH were detected at 1,270 ± 103 µg (g cloth)$^{-1}$ and 233 ± 22 µg (g cloth)$^{-1}$ in Cloth A. 12:2, 14:2, and 16:2 FTOH were not quantitated due to the lack of authentic standards. Total organic fluorine (TOF) measurements of anti-fog sprays ranged from 192 to 21,700 mg/g. FTOHs accounted for a wide range of TOF levels in the 4 anti-fog sprays, ranging from 1-36% by weight, with FTEOs likely accounting for the majority of the remaining TOF content; however, they could not be quantified due to lack of authentic standards. All products tested also included manufacturing byproducts, including fluorotelomer ethers, a fluorotelomer fumarate, and fluorotelomer ethoxylate ethers. Since ethoxylates have been shown to be highly adipogenic in vitro, we decided to test these solutions for adipogenic activity. All four anti-fog sprays exhibited significant cytotoxicity and adipogenic activity (either triglyceride accumulation and/or pre-adipocyte proliferation) in the murine 3T3-L1 cell model. Sprays C and D were the most cytotoxic, with cell viability compromised at concentrations ≥0.1%. Spray A became cytotoxic at 1% and Spray B was only cytotoxic at the highest concentration tested (10%). Three of the four anti-fog sprays exhibited significant triglyceride accumulation at concentrations as low as 0.001%, which is equivalent to approximately 5 nM of the positive control, rosiglitazone. Importantly, this biological activity was observed at an *in vitro* dose that is less than the dose applied to eyeglasses from one pump of the spray based on their densities. All together, these results suggest that more research is needed to understand the health risks from using these PFAS-containing products.

04.04.03 Per- and Polyfluoroalkyl Substances (PFAS) in Children's Products in North America Market

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Per- and polyfluoroalkyl substances (PFAS) have been commercialized since the 1950s and widely used in various applications, such as textile finishing, food packaging, paper finishes, electroplating, and firefighting. Public concern over the health risk posed by PFASs has triggered increasing number of studies conducted to address their environmental occurrence and associated toxic effects. However, knowledge remains limited in children exposure to PFAS. We collected 76 children's products samples from the US and Canadian market ranging from bibs, snow suits, raincoats, mittens, and clothes marketed as stain resistant. We first screened for total fluorine (total F) using particle-included gamma ray emission spectroscopy (PIGE). We then analyzed them for ~50 neutral and ionic PFAS, including perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkyl sulfonic acids (PFSAs), fluoroalkyl sulfonamides (FASAs), fluoroalkyl sulfonamidoethanols (FASEs), fluorotelomer carboxylic acids and fluorotelomer sulfonic acids (FTCAs/FTSAs), fluorotelomer alcohols (FTOHs), fluorotelomer sulfonates (FTSs), perfluoroether sulphonic acids (PFESAs) fluorotelomer acrylates and fluorotelomer methacrylates (FTACs/FTMACs), and polyfluoroalkyl phosphate easters (PAPs). PFAS were detected in all children's textile products from both US and Canadian markets. When selecting products with the highest total F concentrations, median ∑PFASs was 240 and 280 ng/g for US and Canada, respectively. The median total F concentrations were 1740 and 7000 µg/g. The big gap between PIGE and targeted levels was likely due to unknown PFAS compounds, including precursors. The most abundant compounds detected were 6:2-FTOH (median: 445 ng/g) and the corresponding 6:2-FTMAcr (median: 160 ng/g), which is reported here for the first time. Other neutral PFAS like 8:2 FTOH and FTMAcr were also detected but at significantly lower levels (< 50 ng/g). These are the highest levels reported for neutral PFAS in children's products. Among ionic PFAS, short chain compounds like PFPrA and PFPeA were detected in < 10% of the samples at median concentrations of ~ 15 ng/g. The highest ∑PFAS concentrations were found in clothing made of cotton compared with polyester fabric. This study raises concerns over the use of PFAS in children's products, especially those that are worn close to the body and thus have a higher potential for dermal absorption.

04.04.04 Evaluating PFAS Exposure from Fluorinated Waxes Among U.S. Snow Sport Participants

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Performance snow sport waxes, which are applied to the base of skis and snowboards to reduce friction with snow, are one of many categories of products that contain per- and polyfluoroalkyl substances (PFAS). A limited number of studies have documented PFAS in environmental media near ski venues and in blood collected from elite professional wax technicians in Scandinavia. However, no prior studies have evaluated PFAS exposure among snow sport participants outside Scandinavia or in individuals who participate in these sports in recreational or amateur capacities. To better understand the potential that fluorinated waxes are a source of PFAS exposure among U.S. snow sport participants, we measured PFAS in dust collected from workspaces where fluorinated wax application was ongoing. Dust wipe samples contained up to 1.8 µg/g PFAS, with C8-C14 carboxylic acids and GenX contributing most to total PFAS mass. We also surveyed cross country and downhill skiers and snowboarders about wax application practices. We recruited participants during the 2020-2021 winter via regional and national professional organizations, sport clubs, and snowball sampling. Our respondents (n=570) included people ages 7 to 82 years, from 33 states who participate in snow sports at levels ranging from recreational enjoyment through elite competition. Most participants (92%) had applied wax to skis or snowboards and the majority (67%) had applied fluorinated waxes. Frequency of fluorinated wax application varied widely among participants (range=0-2,000; mean=84.5 pairs of skis or snowboards per year). While coaches or other industry professionals reported applying the most fluorinated waxes on an annual basis, we were interested to learn that recreational skiers and snowboarders also apply fluorinated waxes regularly. Further, only 57% of respondents reported using any type of respiratory protection (respirator, dust mask) when applying fluorinated waxes. Collectively, this study highlights the potential for fluorinated wax application to serve as a significant source of PFAS exposure among snow sport participants. Recent media attention has increased public awareness about health risks posed by PFAS and the presence of PFAS in fluorinated waxes. Still, many snow sport participants continue to be exposed to PFAS through their participation. Our study identifies need for interventions to reduce snow sport participants' exposure to PFAS via fluorinated wax use.

04.04.05 Polymeric Layers and Associated Volatile, and Non-Volatile PFAS in New Firefighter Turnout Gear

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Firefighter turnout gear is essential for minimizing occupational exposure to hazardous chemicals during training and fire events. Per-and polyfluoroalkyl substances (PFAS) are observed in firefighter serum and the known exposure pathways may include inhalation, ingestion and dermal exposure. However, limited data exists for non-volatile and volatile PFAS on firefighter turnout gear. Four unused turnout garments, manufactured in 2008 and 2019, each comprised of a thermal liner, moisture barrier, and outer layer (12 individual samples) were included in the study. Bulk characterization of the material by pyrolysis-gas chromatography mass spectrometry was used to differentiate polymeric layers from the non-polymeric layers. The layers were also analyzed for 50 nonvolatile and >1000 suspect PFAS by liquid chromatography-quadrupole time of flight mass spectrometry and 21 volatile PFAS by GC-MS. Particle-induced gamma ray emission (PIGE) and instrumental neutron activation analysis (INAA) were used to measure total fluorine. Observed polymeric layers on the moisture barrier included polytetrafluoroethylene (PTFE) on all outward faced material and para-aramid on the inner faced material. Perfluoroalkyl carboxylates (C4 – C16) were dominated by linear isomers and long chains (e.g., C11, C14). Branched and linear (25:75) perfluorooctane sulfonate were also present in all layers of all four turnout garments. The new turnout garments are treated with a combination of telomer- and electrofluorination-based manufacturing products derived from the PFAS classes detected and isomer ratios. Volatile PFAS analysis measured 6:2-fluorotelomer alcohol (6:2-FTOH) in three garments purchased in 2019, while 8:2- and 10:2-FTOH were only measured in the one garment purchased in 2008. The sum of volatile PFAS was higher in concentration than that of nonvolatile PFAS with the C4 N-methyl perfluorobutane sulfonamidoethanol occurring at the highest frequency and concentration of all the target PFAS investigated. Results from PIGE and INAA were comparable and both showed the highest total fluorine in the moisture barrier layer, followed by the thermal liner and outer layer, with the exception of one thermal layer from a garment purchased in 2019 that was advertised to contain PTFE. The summed molar concentrations of nonvolatile and volatile PFAS only made up a small fraction of total fluorine (0.00013 to 1.44%) associated with the fluoropolymer layers of the turnout garments. New turnout garments, not previously exposed to known occupational sources of PFAS, are potentially a source of firefighter occupational exposure to PFAS.

04.04.06 The Air that We Breathe: Neutral and Volatile Per and Poly Fluorinated Alkyl Substances (PFAS) in Indoor Air

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Most humans have detectable levels of perfluorinated alkyl substances (PFAS) in their blood. Given that we spend at least 90% of our time indoors, it is unsurprising that the most common sources of exposure come from food, water, air, and dust. Neutral, volatile PFAS such as fluorotelomer alcohols (FTOHs), fluorotelomer acrylates (FTAcr), perfluorooctane sulfonamides (FOSAs), and perfluorooctane sulfonamide ethanol (FOSEs) are commonly present in indoor environments and are the primary source of exposure to humans. Therefore, quantifying these compounds and their exposure risk to humans is important. To accurately measure these compounds in different indoor environments, two polyethylene (PE) sheets were validated and employed as passive detection tools in various indoor settings including carpeted kindergartens, an outdoor clothing store, carpet store, and laboratories, classrooms and offices from a university. Passive samplers were paired with Radiello active samplers at an outdoor gear and clothing store to determine the KPEa of each compound. The compounds of interest in this study included 6:2 FTOH, 8:2 FTOH, 10:2 FTOH. 8:2 FTAcr, 10:2 FTAcr, MeFOSA, MeFOSE, EtFOSA, and EtFOSE. Samples were extracted in ethyl acetate and analyzed by gas chromatography-mass spectrometry. Partitioning between both PE sheets suggests that interactions of the passive samplers with the compounds are occurring by absorption. Results indicated that volatile PFAS, in particular FTOHs, are ubiquitous in these environments. For example, in carpeted Californian kindergartens, 6:2 FTOH dominated with concentrations ranging from 9-600 ng m^{-3}, followed by 8:2 FTOH (3 %). When detected, 6:2 FTOH (83%, < MDL – 1900 ng m^{-3}), and 8:2 FTOH (17%, < MDL-270 ng m^{-3}) also dominated total PFAS in the university rooms. At the outdoor clothing store, FTOHs were the most abundant and dominant group, and from these compounds, 8:2 FTOH was the most abundant with an average concentration of ~200 ng m^{-3} followed by 6:2 FTOH and 10:2 FTOH with average concentrations of 70 ng m^{-3} and 30 ng m^{-3} respectively. Volatile PFAS from air, carpet, and dust were closely related to each other, which indicates that carpets and dust are major sources of FTOHs in air. Nonetheless, air poses the largest exposure risk of FTOHs and biotransformed perfluoroalkyl acids (PFAAs) to children age 2 to 6 years old. Thus, this research highlights the need for further regulation of PFAS precursors.

04.04.07 Neutral and Volatile Perfluorinated Alkyl Substances (PFAS) in Air and Water from Dhaka, Bangladesh

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Neutral, volatile perfluorinated alkyl substance (PFAS) precursors, such as fluorotelomer alcohols (FTOHs), perfluorooctane sulfonamides (FOSAs), and perfluorooctane sulfonamide ethanol (FOSEs) are industrial (by)products and have been found in urban, industrial, and even rural locations. Despite becoming a party to the Stockholm Convention in 2007, Bangladesh does not regulate PFAS and has not accepted the amendment listing, and perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), along with other PFAS remain unregulated. Data on PFAS environmental concentrations and emissions, as well as information on human exposure in developing countries is scarce, although desperately needed. To accurately measure these compounds in outdoor environments from urban, rural, and industrial regions from Dhaka, Bangladesh, we proposed the use of apolar polyethylene (PE) sheets as a passive detection tool for air and water. The compounds of interest in this study included 6:2 FTOH, 8:2 FTOH, 10:2 FTOH. 8:2 FTAcr, 10:2 FTAcr, MeFOSA, MeFOSE, EtFOSA, and EtFOSE. The sites were evaluated using two types of pre-cleaned PE passive samplers differentiated by thickness. Samples were extracted with ethyl acetate and analyzed by gas chromatography-mass spectrometry. The most frequently detected compound was 6:2 FTOH ranging from < MDL to 70.00 ng m^{-3} (68%) in air, and from < MDL to 19.00 ng L^{-1} in water (62%), followed by 10:2 FTOH (43% air, 0.68% water) and 8:2 FTOH (35% air, 0.45% water) Outdoor air and water from the textile manufacturing industry in Dhaka, Bangladesh had FTOH concentrations comparable to those found indoors in North America. The latter is significant because indoor air concentrations tend to be twice as high as outdoor air concentrations. Thus, health concerns should be raised given the elevated concentrations found in ambient air and water.

04.04.09 Exposure to Known and Novel PFASs Among Firefighters: Baseline and Post-Fire Serum Profiles

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Firefighters are often exposed to per- and polyfluoroalkyl substances (PFASs) above background concentrations according to previous studies, particularly when the cohorts considered had worked with aqueous film forming foams (AFFFs). In addition to AFFF usage, there are many other occupational PFAS exposure pathways for firefighters (e.g., wearing PFAS-containing turnout gear; entering environments where PFAS-containing materials may be burning). Of course, PFAS exposures that are relevant for the general population (e.g., PFAS-contaminated drinking water; household and dietary exposures) also apply to firefighters. It is essential that we understand the importance of occupational PFAS exposure pathways to determine how firefighting activities contribute to PFAS body burdens and potential health risks. Here, we analyzed paired serum samples from 33 firefighters that were collected annually ("baseline") or 24 - 36 hours after a fire ("post-fire"). Samples were analyzed by high-resolution mass spectrometry (HRMS) to (i) obtain a more comprehensive characterization of PFAS serum profiles in career firefighters, and (ii) determine the impact of firefighting activities on serum PFAS profiles. We developed a streamlined serum extraction protocol for quantitative analysis of 40 PFASs and simultaneous non-target screening. In addition to quantitation of target analytes, suspect screening was conducted for >1000 known and novel PFASs, and semi-quantitative concentrations were estimated for frequently detected novel compounds. Preliminary findings suggest that some perfluoroalkyl sulfonates (PFHxS and branched PFOS) are elevated in this cohort compared to the general population (2017-2018 NHANES), though no pronounced differences were observed between target analyte concentrations in "post-fire" and "baseline" samples. Perfluoroethylcyclohexanesulfonate (PFECHS) and ketone-perfluorooctane sulfonate (K-PFOS) were frequently detected at low concentrations (< 0.2 ng/mL) in both sample groups. Here we will present identification and quantitation of novel and known PFASs detected in this cohort, and discuss what serum PFAS profiles can tell us about potential exposure pathways.

04.04.10 Identification of an Analytical Method Interference for Perfluorobutanoic Acid in Biological Samples

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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 and quantification of novel compounds, low-resolution MS is the more wide-spread and affordable approach for studies examining previously identified PFAS. Low-resolution targeted MS/MS quantification experiments can be highly accurate and precise but are not without potential for interference due to low resolving power of the instrumentation (~5k compared to >60K for high-resolution). Investigations into PFAS in biological matrices has led to the identification of several compounds that mimic and interfere with quantitation of PFAS at low-resolution. For example, taurodeoxycholate (a common bile acid) has been observed to mimic the primary perfluorooctanesulfonic acid (PFOS) MS/MS transition (499→80), while endogenous steroid sulfates has been observed to mimic two of the perfluorohexane sulfonic acid (PFHxS) MS/MS transitions (399→80 and 399→99). As a result, the use of qualifying ion MS/MS transitions are suggested to verify the chemical identity for PFOS and PFHxS, and separation of interferents by liquid chromatography (LC)MS is useful to enhance quantification accuracy. Of note, perfluorobutanoic acid (PFBA) is one of the smaller PFAS observed in biological and environmental samples and has only one MS/MS transition for quantification and none for verification. Recently, our laboratories undertook a targeted investigation into PFAS in the human placenta from high-risk pregnancies utilizing low-resolution, targeted MS/MS. Samples were monitored for a panel of 15 PFAS. Examination of placental samples revealed a widespread (n = 93/122) chemical interferent in the quantitative ion channel for PFBA (213→169). PFBA concentrations were influenced by up to ~ 3 ng/g. Using an hour-long high-performance liquid chromatography (HPLC) method to achieve chromatographic separation the suspect peak eluted close to our isotopically labeled internal standard (IS) for PFBA (Δ Retention Time ≅ 7s). Therefore, HRMS/MS techniques were used to investigate the suspect peak, predict a chemical formula with a score of 99.91, and putatively assign the compound as an oxo-fatty acid with a neutral loss transition.

04.04.11 A Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) Method to Improve Detection of Low Molecular Weight Perfluoroalkyl Ether Carboxylic Acid (PFECA) Isomers

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Per- and polyfluoroalkyl substances (PFAS) are emerging contaminants widely used in a variety of industrial and consumer applications (e.g., fire-fighting foams, household products, etc.). These anthropogenic chemicals persist in various environmental media including water, soil, and air. Due to phasing out legacy PFAS, some manufacturers developed short-chain alternatives like perfluoroalkyl ether carboxylic acids (PFECA), which contain ether bond(s) in the perfluorinated carbon backbone. Published liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods cover a wide range of these replacement chemicals including perfluoro-3-methoxypropanoic acid (PFMPA) and perfluoro-4-methoxybutanoic acid (PFMBA). However, many methods do not monitor for the branched isomers of PFMPA and PFMBA, which are perfluoro-2-(perfluoromethoxy)propanoic acid (PMPA) and perfluoro-2-ethoxypropanoic acid (PEPA), respectively. Although these isomers are chromatographically separable under certain conditions, using the common MS/MS transitions for PFMPA (*m/z* 229 → 85) and PFMBA (*m/z* 279 → 85) will yield low or no detection signal for PMPA and PEPA, thus leading to underestimated values or non-detects. Herein, we developed an LC-MS/MS method that compared various MS/MS transitions for these isomers. We applied the developed method to different matrices including drinking water, serum, and fish liver. Current observations demonstrated that the highest signal is received when using the following mass transitions: PMPA (*m/z* 185 → 85) and PEPA (*m/z* 235 → 135). These MS/MS transitions will improve detection of these isomers and lead to better monitoring and exposure estimates of PFECA in humans and the environment.

04.04.13 Influences of Household Behavior and Demographics on Indoor Air Quality

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Indoor air pollution is ranked among the top five environmental health risks by the EPA. On average, indoor pollutants can be anywhere from two to five times higher than outdoors. Americans spend approximately 90% of their time indoors, with vulnerable populations (children, elderly, individuals with chronic illnesses) spending even more of their time inside. Shifts in building construction, composition of building materials, and increases in use of consumer products indoors has resulted in increased exposure to volatile (VOCs) and semi-volatile organic compounds (SVOCs). Traditionally, indoor air quality research has focused on VOCs or individual classes of SVOCs. However, few studies have investigated a broad range of chemical compounds in indoor air, and the influences of household behavior and location on chemical profiles. Stationary passive air samplers were deployed using community-engaged research at 24 different locations across the United States. Samplers were placed inside and outside the home, and participants completed a survey evaluating household behavior. Samples were analyzed for over 1,530 individual analytes using a semi-quantitative screening method consisting of over twelve different chemical categories. Of the 1,530 analytes in our method, a total of 79 chemicals were detected in at least one sample. Indoor samplers ranged from 12 to 31 detects with an average of 27 detections per sampler. Outdoor samplers ranged from 8 to 33 detects with an average of 15 detections per sampler. For over 50% of chemicals detected both in- and outdoors we found that indoor concentrations were over ten times higher than outdoors. However indoor concentrations were significantly lower for participants who had their windows open compared to those who had their windows closed or had an air conditioning unit running. Future directions of this study include further investigating how specific behaviors or household demographics may influence exposure to certain chemical categories. Indoor air quality continues to be a large area of focus, especially with individuals spending even more time indoors over the past year. Results from this study will help inform influences on chemical exposure profiles and potential interventions that can be taken to help improve indoor air quality.

04.04.14 Determinants of Exposure to Endocrine Disrupting Chemicals Following Hurricane Harvey

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Hurricane Harvey was a category four storm that induced catastrophic flooding in the Houston metropolitan area. Multiple industrial complexes released excess emissions due to unplanned shutdowns or infrastructural damage as a result of the hurricane. Additionally, 13 of the 41 superfund sites within Harris County, Texas flooded. Public health concerns were immediately raised regarding potential exposure to toxic chemicals from the excess industrial releases and flooded Superfund sites. A multi-institutional team formed to deploy personal passive sampling devices in the form of silicone wristbands immediately after the hurricane to determine whether the flood had impacted personal chemical exposure. Participants (n=173) wore a wristband for seven days and completed a questionnaire to determine various flood-related demographic variables. This included information necessary to calculate an Area Deprivation Index (ADI), which was used to measure a participant's socioeconomic status as well as gage exposure to flooding (i.e. home flooding status, amount of time spent in a flooded home, participation in flood clean-up activities, and degree of flooding in home). We analyzed each wristband for 1,530 chemicals. Additionally, we compared the list of chemicals we analyzed to the United Nations and Danish EPA list of endocrine-disrupting chemicals (EDCs). Similar exposure profiles to EDCs existed across participants, independent of home flooding or flood clean up status, and flood amount in home. The most profound finding related to flood status is that participants who remained in flood-damaged homes during remediation had higher levels of exposure to multiple EDCs including lilial, triphenyl phosphate (TPP), and bis(2-ethylhexyl) phthalate compared to participants who moved after home flooding. Most of the variables studied that were associated with EDC exposure were demographic (i.e. participant ADI, race/ethnicity, and participant neighborhood). A high ADI (low socioeconomic status), identifying as Black/African American or Latino, and certain neighborhoods were all associated with increased exposure to EDC's in general and the specific EDCs lilial, TPP, butylated hydroxytoluene, butyl benzyl phthalate, and bis(2-ethylhexyl) phthalate. Moderate collinearity exists between these demographic variables and time spent in a flooded home where individuals living in lower-income neighborhoods were more likely to remain in their flood-damaged homes during remediation.

04.04.15 The Role of Chemical Properties in Human Exposure to Organic Contaminants

Z. Zhang, University of Nevada, Reno / Environmental Science; S. Wang, L. Li, University of Nevada, Reno / Community Health Sciences

One of the ultimate goals of environmental exposure sciences is to understand the mechanisms, pathways, and determinants of human exposure to the wide spectrum of environmental contaminants. Chemical properties, e.g., chemicals' partitioning, reaction, and dissociation behavior in the environment, play a central role in controlling human exposure to environmental contaminants. This presentation will provide the audience with systematic, mechanistic insights into the human intake and absorption of various organic contaminants via oral ingestion, dermal permeation, and respiratory inhalation. We will comprehensively analyze the combinations of chemical partitioning, reaction, and dissociation properties that lead to the high susceptibility of human exposure, as well as the relative importance of exposure routes between chemicals with different properties. Our analysis indicates that, in general, hydrophobic, recalcitrant chemicals of relatively low volatility tend to accumulate in animal- or plant-based foods and be ingested by humans through diet. Dietary ingestion becomes less influential for more degradable chemicals. When used indoors, poorly volatile chemicals are predominantly distributed on indoor surfaces and dust; surface-to-hand and subsequent hand-to-mouth contact is the main mechanism responsible for ingestion of these chemicals. The ingested chemicals can be absorbed into the circulatory system if they are neither too hydrophobic nor hydrophilic and possess relatively small molecular sizes. In the meanwhile, human skin can absorb volatile chemicals directly from air, poorly volatile chemicals bound to dust on hands or present as surface residuals, as well as various chemicals intentionally applied to the skin. Dermal absorption becomes highly efficient when a chemical has moderate hydrophobicity and a relatively small molecular size. Further, inhalation matters for relatively volatile chemicals, among which hydrophilic chemicals can be efficiently absorbed by the respiratory tract. This presentation provides theoretical information for the effective identification of the most relevant exposure pathways for chemicals with different properties, which guides exposure-oriented biomonitoring efforts and the design of safe and sustainable chemical substances.

04.04.16 How Confident Can Current Computational Models Predict Fate and Exposure for the Myriad Chemical Substances on the Market?

Z. Zhang, University of Nevada, Reno / Environmental Science; L. Li, University of Nevada, Reno / Community Health Sciences

More than 350,000 chemicals and mixtures have been registered in national and regional chemical inventories. It is critical to evaluate whether their production and use pose potential risks to ecological and human health. Generic fate and exposure models, i.e., models building on mechanistic descriptions of physical, chemical, physiological, and behavioral processes and not specific to certain categories of chemicals, are cost-effective and user-friendly tools for this goal. Excellent examples of these models, e.g., EUSES, PROTEX, USEtox, RAIDAR(ICE), have been used to track the fate and behavior of thousands of chemicals throughout the entire continuum from chemical production to dose in the human body. However, despite being largely mechanistic or semi-mechanistic, these models are often parameterized based on empirical relationships generalized from experimental measurements. The physicochemical space of the training set, i.e., the applicability domain, is therefore critical to determine whether an empirical relationship is applicable to make defensible predictions for chemicals. The models also rely on computational tools such as quantitative structure-activity relationships (QSAR) for parameterization. Meanwhile, assumptions used in the models, e.g., well-mixed homogenous environmental compartments, steady state, and equilibrium partition between sub-compartments, are an important determinant for a model's applicability to certain chemicals. In this presentation, we evaluate the extent to which existing generic fate and exposure models are applicable to tens of thousands of chemicals in different national and regional chemical inventories. Specifically, using the state-of-the-art QSAR tools, we parameterize the models to predict exposures of ecological receptors and humans to this myriad of chemicals. We systematically analyze the applicability domains of built-in empirical relationships, parameterization tools, and model assumptions for these chemicals. We then determine the chemicals in these inventories for which these models can make predictions with the highest confidence. Preliminary results indicate that only a limited number of chemicals in these inventories fall entirely within the applicability domains of the current models. This presentation, for the first time, systematically informs academia and regulatory agencies on the applicability and uncertainty of the generic fate and exposure models.

04.04.17 Assessing Children's Exposure to Novel Flame Retardants in House Dust: A Case Study

N. DeLuca, E. Cohen Hubal, U.S. Environmental Protection Agency / Center for Public Health and Environmental Assessment

House dust is regularly analyzed to detect the presence of harmful chemicals in the residential environment. Because children's behaviors cause them to more frequently come in contact with house dust on floors and near-floor surfaces, there is higher potential for exposure to chemicals in the house dust. In this work, we conduct a children's exposure assessment to novel brominated flame retardants in house dust using recently published literature that measures these emerging chemicals in U.S. homes. Our case study focuses on three child lifestages that span ages 1 year to 6 years, when children are particularly vulnerable to the ingestion of brominated flame retardant chemicals in house dust. Average daily exposure estimates from dust ingestion are calculated for each lifestage, including an average exposure estimate as well as a worst-case exposure estimate. The results of the case study serve to provide a baseline for exposures to novel flame retardant chemicals that have not been included in previous national exposure assessments. Updated exposure estimates for legacy flame retardants are also included for comparison and provide insight on trends in children's exposures over the last decade. Critical data gaps encountered during the analysis identify future research needs, such as additional measurements of novel flame retardant concentrations in house dust, biomonitoring studies in the general population and children, and toxicology studies focusing novel brominated flame retardants that would facilitate the expansion of this case study into a risk assessment.

04.04.18 Towards an Integrated Approach for Studying Contaminants in Air Under the Gaps-Megacities Project

A. Saini, T. Harner, Q. Liu, Environment and Climate Change Canada / Air Quality Research Division; X. Zhang, Concordia University / Department of Chemistry and Biochemistry; L. Li, University of Nevada, Reno / School of Community Health Sciences; J. Liggio, Environment and Climate Change Canada / Air Quality Research Division; S. Halappanavar, D. Wu, Health Canada / Mechanistic Studies Division

The Global Atmospheric Passive Sampling –Megacities (GAPS-MC) study was initiated in 2018 with the aim to monitor atmospheric contaminants in the urban environment and linking them to associated health risks. Under the pilot phase of the GAPS-MC study, passive air samples were collected for two consecutive periods of 3 months each at 20 mega/major cities around the globe. Analysis was performed for a range of persistent organic pollutants (POPs) and contaminants of emerging concern such as organophosphate esters (OPEs) and novel flame retardants (NFRs). OPEs were 2 -5 orders of magnitude higher in air compared to other FRs and exhibited a moderately strong and significant correlation with the Gross Domestic Product of the cities, which is related to the use of modern commercial products (that contain and emit these chemicals). *In vitro* toxicity screening of the air extracts from previously codeployed PUFs in Toronto (the only GAPS-MC station having multiple sites) showed an increased reactive oxygen species (ROS) formation and changes in the expression of multiple pro-inflammatory mediators in immortalised adenocarcinomic human alveolar basal epithelial cells (A549) post-exposure to air extracts. While the unsubstituted parent Polycyclic Aromatic Hydrocarbons (PAHs) were associated with the various toxicological endpoints, contributions of nitro- and oxy-PAHs during the springtime were also identified as important contributors to observed toxicity. Despite the low concentrations of nitro- and oxy-PAHs relative to parent PAHs (in particular the 16 PAHs that are prioritized by USEPA), it is evident from the *in vitro* toxicity screening results that the former also exhibits potential to induce harm, this is an area warranting further research. Lastly, non-target suspect screening of GAPS-MC extracts also uncovered transformation products of OPEs in the air mixture, which were more toxic and persistent than the parent chemicals. The GAPS-MC project is an excellent platform for studying mixtures of priority chemicals and their transformation products in ambient air with the aim to better understand the implications for human exposure and health.

04.04.19 Insecticide and Fungicide Residue Release and Exposure from Treated Seed Recycling at Nebraska Bioethanol Facility

D.D. Snow, University of Nebraska / School of Natural Resources; S. Bartelt-Hunt, University of Nebraska, Lincoln / Civil and Environmental Engineering; J. Wu-Smart, University of Nebraska / Department of Entomology; E. Van Wormer, University of Nebraska / School of Natural Resources

Outdated seed corn coated with neonicotinoid insecticides and fungicides has been recycled and used as a feedstock at a bioethanol facility in eastern Nebraska since 2015. This facility was one of only two plants receiving treated seed and reportedly processed over 98% of expired seed in the United States. Numerous environmental violations led to an emergency order to cease operations by the state of Nebraska in February 2021. Rupture and runoff onto adjacent properties and leakage of wastewater contaminant lagoons led to additional sampling showing alarmingly high levels of multiple insecticides and fungicides were leaving site storage areas. Laboratory analysis of wastewater and stockpiled distillers grain contained several thousand parts per billion of the neonicotinoid insecticides clothianadin and and thiomethoxam. Grab samples of wastewater run-off in local stream channels contained clothiandin, imidocloprid-desnitro, imidicloprid-urea, metalaxyl, thimethoxam, and thiomethoxam-urea at concentrations up to several thousand part per billion, many orders of magnitude above aquatic life benchmarks. A review of regulatory reports show that wastewater and stockpiled distillers grain were stockpiled and applied annually to surrounding cropland, further dispersing the contamination and leading to multiple complaints by area residents of the odor. Area residents reported exposure from stockpiles and multiple incidents of animal illness and potentially-affected wildlife. University of Nebraska honeybee research areas were severely impacted by exposure to dust, water and plants highly contaminated with parent pesticides and degradation products. Current surface water monitoring shows that highly mobile and persistent neonicotinoid residues and degradation products are at concentrations well above nonpoint source level downstream of the affected watershed months after the run-off event. Ongoing monitoring at this site will provide new insight about the long term fate and potential exposure to elevated levels of pesticides and degradation products used in agriculture.

04.04.20 Using Silicone Passive Samplers to Evaluate Pesticide Exposures in Humans and Pet Dogs: A Comparative Exposure Assessment

C. Wise, S. Hammel, Duke University / Nicholas School of Environment; N.J. Herkert, Duke University / Civil and Environmental Engineering; M. Breen, North Carolina State University / Molecular Biomedical Sciences; H.M. Stapleton, Duke University / Nicholas School of the Environment

People are chronically exposed to various pesticides through the diet, but also through herbicide applications in lawns and pesticide treatments around the home. Chronic household exposure to pesticides affects people and their pets, and some studies suggest pesticide exposure in dogs may be associated with cancer. Companion animals are increasingly recognized for their value in comparative health studies, and their shared daily environment with people suggests they may be valuable in supporting environmental health research. In this study, we used wearable silicone passive samplers to support a comparative exposure assessment. We recruited 30 people and their pet dogs (living in the same household) to participate in a study to determine how well silicone wristbands (for human) and dog tags (worn on dog collars) can predict urinary pesticide biomarkers of exposure. Participants wore the silicone samplers for 5 days. They collected urine from themselves and their dogs on Days 1, 3 and 5 of the study. Urine samples were pooled for analysis of pesticide biomarkers. Using targeted GC-MS analyses, we quantified 8 pesticides in silicone samplers. Using a suspect screening approach, we additionally identified N,N-diethyl-m-toluamide (DEET), promecarb, flamprop-methyl and fipronil on the silicone samplers with high detection frequencies, and several had statistically significant correlations between wristbands and dog tags (r_s=0.67-086, $p< 0.01$). Pooled urine samples were quantified for 15 pesticide metabolite biomarkers. Several pesticides, including permethrin, DEET and chlorpyrifos, were detected with high frequency (>70%) in wristbands and urine of both humans and dogs, as corresponding biomarkers. Compared to adults evaluated in the U.S. general population, these dog-owners had higher urinary pesticide metabolite concentrations. Significant and positive correlations were observed between silicone sampler levels of permethrin and DEET with their corresponding urinary metabolites (r_s= 0.50-0.96, $p< 0.05$) in both humans and dogs. Owners that reported using flea and tick products containing fipronil on their dog had significantly higher levels of fipronil in wristbands (~10X) and dog tags (~100X) compared to those who did not ($p< 0.01$). This study demonstrates that pet dogs can act as proxies for human pesticide exposures in the home environment, potentially providing a new way to study relationships between environmental exposures and disease etiology.

04.04.21 Human Exposure to Pesticides in Residential Buildings

S. Vaezafsh, University of Toronto / Earth Science; Y. Wan, University of Toronto / Physical and Environmental Sciences; J.A. Siegel, University of Toronto / Civil and Mineral Engineering; L. Jantunen, Environment and Climate Change Canada / Air Quality Processes Research Section; M.L. Diamond, University of Toronto / Department of Earth Sciences

Humans are exposed to elevated pesticide levels in indoor environments due to the high persistence of these chemicals in the absence of sunlight, moisture, and microbial degradation. There is a concern regarding human exposure to pesticides, especially for pregnant women and children, due to reported potential health impacts. Pesticides are used In indoor environments for controlling vermin (e.g. allethrin and cyfluthrin), are used for pet protection (e.g., permethrin), are contained in some treated consumer products (e.g. imidacloprid and chlorothalonil) and building materials (e.g. azoxystrobin, fluoxastrobin, dazomet, etc.). Pesticides can also enter from outdoors, especially in agricultural areas. Typical assessments of pesticide exposure have not considered contributions from indoor environments to be significant. In Canada, pyrethroid insecticides are the first choice for pest control in residential buildings. While pyrethroids are active ingredients in many insecticidal products In Canada, no data are available about their concentrations indoors. As such, our research concerns the determination of concentrations of pyrethroid insecticides (e.g. allethrin, permethrin, Pralletrine, etc.) and fungicide (e.g. azoxystrobin, fluoxastrobin, pyraclostrobin, etc.) as well as chlorothalonil, malathion, and imidacloprid in residential buildings. Pesticide levels were measured in indoor air samples collected using passive silicone rubber (polydimethylsiloxane or PDMS) air samplers from residential buildingsthrough two previously conducted projects with very different household socioeconomic statuses. Results will be presented showing pesticide levels in indoor residential air and the influence of several study factors on pesticide concentrations.

04.04.23 Wildfire Impacts on Indoor and Outdoor Air Quality

C.C. Ghetu, Oregon State University / Environmental and Molecular Toxicology; D. Rohlman, Oregon State University / College of Public Health and Human Sciences; B.W. Smith, R. Scott, P. Hoffman, K. Adams, K.A. Anderson, Oregon State University / Environmental and Molecular Toxicology

This study focused on the development and testing of a community engaged approach to better understand the fate of polycyclic aromatic hydrocarbons (PAHs) from wildfires, and the potential for human exposure. Wildfire smoke is a complex mixture of particulates, volatile and semi-volatile organic compounds and inorganic compounds. Air quality estimates, used to inform public health recommendations, are based on outdoor stationary monitors, and may not reflect indoor air concentrations. Additionally, current public health recommendations are based on risks to particulate matter and other regulated chemicals. Thus, these values may not reflect indoor air concentrations, nor chemicals present in the vapor phase. Using a community-engaged research approach, passive sampling devices were deployed indoors and outdoors at fifteen locations across Washington, Idaho, California and Oregon during the wildfire season (August – November) in 2018, 2019 and 2020. Wildfires led to significant increases in 4, 5 and 6 ring PAH air concentrations outdoors. However, wildfires did not impact indoor air concentrations as significantly as outdoor air concentrations. Wildfires also led to higher inhalation cancer risk indoors and outdoors. On average, indoor air quality was worse than outdoor air quality except when the Air Quality Index (AQI) approached and/or exceeded 125 (unhealthy for sensitive groups). On average, above an AQI of 125 outdoor air PAH concentrations become similar or worse than indoor air. Forensic sourcing of indoor PAHs suggests different exposure profiles between indoor and outdoor air during and after wildfire activity. Personal behaviors such as opening and closing windows may influence PAH exposure. These conclusions suggest that vapor-phase chemicals and indoor air quality needs to be considered for public health recommendations related to wildfire activity.

04.04.25 Discovery of Nitrobisphenol a as a Ubiquitous Indoor Estrogen Related Receptor Gamma Ligand

D. Yang, University of Toronto / Department of Chemistry; J. Liu, University of Toronto; S. Wang, H. Peng, University of Toronto / Department of Chemistry

Estrogen related receptor gamma (ERRg), an orphan nuclear receptor regulating human cellular energy metabolism, has been associated with multiple metabolic diseases, such as hyperglycemia, insulin resistance and alcoholic liver injury. While Bisphenol A (BPA) is a well-known ligand of ERRγ, the potential existence of other unknown ligands were explored in this study. We employed the protein affinity pulldown assay (PUCA), to identify ERRγ ligands from Canadian house dusts in an unbiased way. The recombinant His-tagged ERRγ LBD was overexpressed in *E. coli* with good yields, and was used to isolate its ligands from pooled indoor dust extracts. The isolated ligands were subjected to non-targeted analysis, and two molecules were identified with high selectivity. While one of the ligands was identified as BPA, the other top hit was a previously unknown compound and was predicted as nitro-BPA according to retention time and MS^2 spectra. Its identity was further confirmed by a lab synthesized standard. The nM range binding affinity of nitro-BPA to ERRγ was supported by protein-based and reporter cell based bioassays. We further analyzed nitro-BPA in 33 Canadian house dusts, and nitro-BPA was detected in the 31 out of 33 house dust samples, demonstrating the ubiquity and abundance of the compound in the indoor environment. Note that nitro-BPA is not produced by any known industrial sources. While the project is still ongoing, compelling evidence was merged to support that nitro-BPA is formed from native BPA via indoor transformations. This study discovered a new ERRg ligand, and highlighted that nitration might be a previously ignored indoor transformation pathway for bisphenols, through which the chemicals produced might be a great concern to human health.

04.04.26 Fast Validated Direct Injection Determination of HAAs in Drinking Water with IC-MS/MS Per EPA 557

P. Voelker, ThermoFisher Scientific

Haloacetic acids (HAAs) are among the disinfection byproducts produced during chlorination of water which contains natural organic matter and bromide. EPA method EPA 552.3 using GC-ECD is challenging and time consuming, requiring extraction and derivatization that can take up to 4 hours. Ion chromatography-mass spectrometry (IC-MS/MS), per EPA Method 557 offers a fast, sensitive, and selective alternative that does not require sample pretreatment. Excellent peak resolution and linearity are achieved for analyte concentrations between 0.4 and 100 µg/L in a high fortified matrix. The detection limit is less than 0.4 µg/L for each of the five regulated HAAs and less than 1 µg/L for the other four. Results using IC-MS/MS show HAA9, Bromate, and Dalapon can be analyzed in 35 min using Thermo Scientific™ Dionex™ IonPac™ AS31 anion exchange column. To demonstrate (IC-MS/MS) system to system variability, results from a repeatability study of multiple systems are presented using HAA9, Bromate, and Dalapon.

04.05 Microplastics Research Priorities: Detection, Analysis and Effects

04.05.01 Quality Assurance/Quality Control in Microplastics Processing and Enumeration

M. Kosuth, University of Minnesota / Environmental Health Sciences; M. Kosuth, Dunwoody College of Technology / Arts & Sciences; M.F. Simcik, University of Minnesota / School of Public Health

Despite six decades of microplastic contamination research, this field struggles to establish universally accepted methods and techniques. Furthermore, scant published work scrutinizes the application, effectiveness, and utility of positive and negative controls. The present study examines three common practices in microplastic processing and enumeration. The first component evaluates four filtered water sources commonly used to run field and procedural lab blanks, which are essential in accounting for particles unintentionally introduced during sample handling. Preliminary work reveals significant variation between filtered waters, which suggest the magnitude of correction applied to samples is dependent on the type of filtered water chosen for blanks. The second component chronicles particle loss, specifically particle adhesion to the filtration apparatus. Water samples spiked with a plastic standard and vacuum filtered through a two-piece borosilicate glass filtration apparatus yielded different recovery rates than those filtered through a two-piece apparatus made of stainless steel. The steel filter had significantly higher recovery rates of polyethylene fragments compared to glass, but slightly lower recovery rates of nylon fibers, while the recovery rates for polyester film were comparable. The final component compares the effectiveness of a frequently used imaging software called ImageJ with a new, customized program designed by MIPAR. Both systems analyzed identical images captured from a set of PCTE filters that contained either filtered water or an environmental media spiked with the aforementioned plastic standard. While ImageJ is capable of particle enumeration as well as basic sizing and categorization (fiber, fragment, particle) it grossly overestimated particles and often mischaracterized fragments and fibers. Particle count summaries from MIPAR, however, were in accord with the known quantity of standard in each sample. These findings underscore the importance of running positive controls when using new techniques or even refining established techniques. This work is essential to reporting accurate results in microplastics contamination research.

04.05.02 Source-Specific Categorization of Microplastics in Surface Waters of the Great Lakes

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Microplastics enter the North American Great Lakes from a variety of sources which are not well understood. Particle morphologies can be a useful indicator of microplastic sources. Motivated by source reduction, we first assess the sufficiency of categories for reporting microplastics and then augment the current categorization scheme by introducing a source-specific categorization framework. We apply the source-specific categorization framework to 62 surface water samples collected in 2018-19 from nearshore sites in Lakes Ontario, Erie, and Superior using manta trawls. Results show greatest abundances in Lake Ontario, specifically at sites near the Greater Toronto Area, followed by Lake Erie and Lake Superior, respectively. While fragments dominated all three lakes, patterns in particle morphologies suggest different source contributions between the lakes. Particle morphologies were similar in Lakes Ontario and Erie but differed from Lake Superior. In Lakes Ontario and Erie, particle morphologies were more diverse, largely dominated by commercial and consumer sources (e.g. spherical and irregular microbeads, commercial fragments, and polystyrene foam). In Lake Superior, polyurethane foams were a distinct morphology, not previously reported in microplastic studies in the Great Lakes region. Our results highlight that particle morphology can be used to refine categories to be more source-driven. Such specific information allows for more direct assessment of source contributions and focusing of reduction efforts. Future studies should consider using refined categories to support source apportionment efforts.

04.05.03 A Novel Methodology for the Removal of Microplastics from Marine Sediments

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Microplastics have become ubiquitous throughout the environment in all types of ecosystems. To understand plastic quantities and the polymer compositions present in the environment, the microplastic must first be extracted from these matrices. This is a feat that is currently lacking efficiency, reproducibility, and standardization throughout the field of microplastic research. Current methods for removing microplastics from the environment use inefficient glassware and expensive density separation devices that require transferring samples between glassware, with each transfer increasing the likelihood of sample loss. To remove the organic matter in a sediment sample, three chemicals are commonly used including Fenton's reagent (hydrogen peroxide and an iron catalyst), sodium hydroxide, and potassium hydroxide. However, these methods are quite harsh and damaging, potentially leading to the loss or misidentification of the microplastics. The objective of our study is to design a readily accessible and affordable sediment density separation device (DSD) to optimize and harmonize research in the microplastics community. A DSD was built with two 1.5 inch diameter sight glasses connected by a stainless steel ball valve. In a novel approach to dealing with organic matter in the sample, a vacuum is applied prior to density separation which reduces the amount of organic plant matter in the sample by 93 %. Stainless steel balls are used inside the DSD to mix only the bottom in order to release plastic particles bound to sediment and increase recovery. Furthermore, all steps of the separation process, until filtering, take place inside the DSD, eliminating the need for sample transfer and greatly reducing the chances for sample loss. The recovery potential of our DSD is being tested by spiking a sample of deep-sea sediment in triplicate with eight polymers (polypropylene, high-density polyethylene, polystyrene, nylon-6, polyvinyl chloride, crumb rubber from tires, and two fibers, polyester and cellulose acetate) between 100 and 300 µm in size. Separation is achieved with 2.0 g/mL sodium polytungstate, of which >80% can be recycled. The count and polymer identity will be compared before and after separation using a microscope FTIR. The acceptable range of recovery from the DSD will be 70 % - 130 %. Results from this novel apparatus will be presented.

04.05.05 Seasonal Variations in Microplastic Form and Concentration Between a Historically Polluted and Relatively Pristine Lake

L. Markley, C.T. Driscoll, A. Costello Staniec, Syracuse University / Civil and Environmental Engineering

Plastic pollution is a widespread issue impacting freshwater ecosystems, which may vary regionally with respect to the sources and pathways of microplastics (< 5 mm). Microplastics are diverse contaminants that can vary in form (size, shape, color, polymer type) and concentration both spatially and temporally, with additional variations between sampling methods. This work, funded by the USGS, aimed to characterize microplastic contamination in Onondaga and Skaneateles Lakes in Central New York with seasonal sampling over a year using a combination of bulk and volume-reduced sampling methods. Onondaga Lake is historically contaminated and has multiple potential pathways of microplastic from combined sewer overflow (CSO) events, wastewater treatment effluent, and runoff from urban Syracuse. In contrast, Skaneateles Lake serves as the source of unfiltered drinking water for the city of Syracuse and is a relatively pristine body of water. Preliminary results found that Onondaga Lake had higher concentrations of microparticles than Skaneateles Lake, likely owing to more prevalent sources of plastic pollution. Our methodological development found that net samples had a lower concentration, but greater morphological diversity compared to lower volume grab samples. This research is the first looking at microplastics in these lakes, which are important water resources for the surrounding region. These results will further inform future priorities in sampling methodologies and source attribution in freshwater microplastic research.

04.05.06 Microplastic Abundance in Marine Sediments Off the Coasts of Maine and New Hampshire, United States

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Microplastics (MP) (< 5 mm) are ubiquitous in the environment and pose unique toxicological challenges because of their small size, measurement difficulties, and polymeric diversity, leading to uncertainties in our understanding of their fate and potential for adverse impacts. Quantification and identification of MP are crucial to better understanding their impacts. Studies suggest marine MP settle into sediments due to weathering, biofouling, aggregation, ingestion, and other biotic and abiotic processes; therefore, MP abundance may have serious implications for many marine species. The objective of this study is to quantify and identify MP particles between 45 – 1000 µm in coastal sediments using a novel extraction method and Raman spectroscopy, to compare MP abundance among sites with varying degrees of human disturbance, and to correlate MP abundances with other sediment measures. Marine sediment samples were collected off the coasts of Maine and New Hampshire by the U.S. Environmental Protection Agency's National Coastal Condition Assessment (NCCA) program during the 2020 field season and a subset (n=10) was chosen for MP abundance. NCCA samples were also examined for a wide variety of con-commitment measures, such as sediment toxicity, benthic diversity, and chemical contamination, which are valuable supplementary data for this type of study. MP sampling sites were selected based on proximity to areas of high human development (i.e. Portsmouth, NH; Portland, ME), as they are possible land-based sources of plastics, and then chosen to represent varying levels of human disturbance. A novel hybrid method was utilized to extract MPs from the sediments, followed by polymer identification using Raman spectroscopy. This research attempts to broaden our understanding of MP abundance and polymer composition in marine sediments to identify potential sources and to better understand the relationship between MP abundance and other sediment measures.

04.05.07 Microplastic Accumulation and Impacts on Eelgrass (Zostera marina) Ecosystems in Coastal Massachusetts, USA

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Marine microplastic contamination is a global phenomenon which disproportionately impacts nearshore systems such as seagrass meadows. Since their discovery on seagrasses in 2018, there have been several documentations of microplastics within these systems, however much remains unknown regarding their mechanisms and patterns of accumulation, as well as subsequent impacts on seagrasses themselves, associated epiphytes, and surrounding sediment communities. This study presents microplastic densities on eelgrass blades and within the associated water column and sediment at ten sites across Massachusetts of varying human development, to identify accumulation patterns, and propose a mechanism for microplastic aggregation on blades. Furthermore, we synthesize and highlight existing relevant literature regarding potential microplastic impacts on the system and point towards the current gaps and opportunities, noting the critical ecological consequences of these emergent anthropogenic interactions. Analysis shows that microplastic accumulation within eelgrass systems is similar across sites despite level of development and that epiphytes work mechanistically as a trap for microplastics on eelgrass blades. Potential impacts of this accumulation include photosynthesis and growth reduction of epiphytes and seagrasses, sediment characteristic alteration, ecotoxicity due to vectored toxins, and nutrient cycle hindrance. By establishing a basis of knowledge on microplastic accumulation patterns and mechanisms, as well as effects within seagrass ecosystems, we intend to encourage future investigation within this field, as to aid in understanding and reconciling the global microplastic crisis.

04.05.08 A Novel Analytical Approach to Identifying Nanoplastics by Assessing Spatiotemporal Deformation Characteristics with Scanning Electron Microscopy

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With annual plastic production exceeding 335 million metric tons worldwide, plastic waste continues to accumulate in the environment where it degrades into smaller plastic particles over time. Due to their size, nanoscale plastic particles (nanoplastics) may exist in quantities several orders of magnitude greater than those found for microplastics, yet their collective mass may remain well under the limits of detection for most existing analytical methods. The goal of this research is to adapt existing tools to address the analytical challenges posed by nanoplastics identification. Given the thermodynamic properties available from polymer manufacturing processes, we hypothesized that nanoplastics could be differentiated by polymer type using spatiotemporal deformation data collected with scanning electron microscopy (SEM). We selected polyvinyl chloride (PVC), polyethylene terephthalate (PET), and high-density polyethylene (HDPE) to capture the range of thermodynamic properties and molecular structure encompassed by commercially available plastics. Pristine samples of each polymer type were chosen and individually milled to generate micro- and nanoscale particles for SEM analysis. Samples for SEM analysis were prepared uncoated to enable observation of polymer deformation under set electron beam parameters. For each sample type, five particles approximately 1 micrometer in diameter were chosen and videos of particle deformation between 30 and 60 seconds in length were recorded and studied. Preliminary data showed differences in degradation patterns between plastic particles sourced from different commercial polymer types. Further study on this data aims to implement a machine learning algorithm to correctly identify individual nanoplastic particles by polymer type using this data.

04.05.09 Eco-Corona Formation on Plastic: Effect of Plastic Type and Aging

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Concerns about the adverse environmental implications of nano- and microplastic are continuously increasing, yet the understanding of plastic fate in freshwater environments is still limited. Plastic chemistry, size, shape, density as well as the freshwater composition can influence plastic particle fate, transport and biological uptake. Aging of plastics, either by photochemical weathering or by adsorption of organic biomacromolecules which leads to formation of an ecocorona, can further change the physicochemical properties of plastics. However, the impact(s) of photochemical weathering on the physicochemical properties of plastic is often neglected, despite the facts that these may influence the adsorption of organic biomacromolecules onto the plastics. Here results of systematic studies on adsorption of a selection of ubiquitous organic biomacromolecules (humic & fulvic acids) to a set of the most environmentally prevalent polymer types (polyethylene, polyethylene terephtalate, polypropylene & polystyrene) in their pristine and photochemically weathered forms, using Quartz Crystal Microbalance with Dissipation (QCM-D) as analytical tool. QCM-D allows for determination of the mass of molecules on the surface of the quartz crystal. We spin coated various polymers onto the QCM-D sensor surface followed by passing solutions of organic biomacromolecules dissolved in synthetic freshwater over the polymer coated quartz crystals to monitor adsorption over time, as measured by changes in the resonance frequency of the crystal. The extent of ecocorona formation on pristine plastics was compared to plastic films which underwent photochemical weathering. Both pristine and UV aged plastic films were characterized before ecocorona formation by contact angle measurments (changes in polarity), Fourier Transfer Infrared spectroscopy (chemical changes) and Atomic Force Microscopy for changes in physical surface roughness. It is anticipated that photochemical weathering of the pristine polymer surface will increase its polarity and result in decreased adsorption of more apolar organic biomacromolecules in synthetic freshwater. This information will help to understand the rate and extent of ecocorona formation for dissolved organic matter on different pristine and photochemically weathered plastics. Ultimately, this will allow us to produce more realistically aged materials to be used in further experiments on fate, transport and biological uptake of plastics.

04.05.10 Influence of Microplastics and Freshwater Snow Heteroaggregation on Particle Settling Rates in the Freshwater Environment

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To date, many studies have focused on the detection and quantification of microplastics (MPs) in natural waters, but just a few have been conducted to systematically understand the processes which drive MPs fate and transport in freshwater ecosystems. Freshwater snow (FWS), a mixture of algae and natural particles, is responsible for the flux of organic matter from the water surface to the sediment and can potentially act as a vector for MPs through the water column. The heteroaggregation between MPs and FWS can impact the settling rates and transport of both constituents, which may change pollution pathways (for the MPs) and nutrient cycling (for the FWS). Here, we focused on the settling rate of MPs in freshwater in comparison to FWS, as well as when MPs and FWS form heteroaggregates. Metal-doped plastics of different density (PET, PLA), size classes (63-125, 125-250 µm) and morphologies (fibers, fragments) were tested to determine which parameter(s) influence MPs settling rates the most. FWS was created by mixing freshwater algae (Asterionella, Mycrocistis, Kirchneriella) on a roller table to simulate the formation of natural flocks over time. Heteroaggregates of FWS and MP were made by adding fixed amounts of MPs and algae cells and mixing on a roller table. We systematically analysed the settling of particles in a plexiglass column (150 cm tall) filled with synthetic freshwater, illuminated by a laser to track particles with a series of cameras vertically aligned with the column. Image sequences were acquired and analyzed with a tracking software to determine settling rates. Three tests were performed: MPs of various composition, FWS and MPs-FWS heteroaggregates. To disentangle the settling rates of MPs when incorporated into aggregates, sequential samples were taken over the depth of the column to further analyse the settling material. Here, the use of metal-doped MPs, which can be quantified by ICP-MS, made analysis of the plastic easier. While data analysis is still ongoing, we hypothesize that MPs size and shape and incorporation into FWS will be important variables in changing the settling dynamics of the materials. Collectively, we provide further insights on the settling rates of MPs which can be used as input value for future fate models in freshwater systems, as well as an indication as to how the presence of MPs can impact the biogeochemical cycles through altering FWS settling in freshwater.

04.05.12 Influence of Water Temperature and UV-Induced Weathering on Release of Polymer Additives from Microplastic Fibers

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Microplastic fibers can constitute up to 90% of synthetic particles found in ambient waters, yet few laboratory-controlled microplastic fate studies have examined this particle type. Environmental release of synthetic fibers can occur through atmospheric deposition or discharge of waste/stormwater, after which the particles are subject to environmental weathering. UV exposure can change the polymer surface morphology which may impact release of polymer additives. Plastic-based textiles use a suite of chemical additives to enhance the functionality, water-resistance, and coloring of the material —such as plasticizers, per- and poly fluoroalkyl substances (PFAS), and disperse azobenzene dyes (azo dyes)— many of which are suspected endocrine disrupters and toxicants. We hypothesize that UV-weathered microplastic fibers will release a different suite of chemical additives than untreated fibers, and increased water temperature will increase the rate of additive release to freshwater. To test this hypothesis, polyester (PES) fibers were examined for additive desorption in freshwater with two independent variables: water temperature and UV pre-exposure. PES fibers were generated from a water-repellant, black fleece garment purchased on Amazon and underwent ethyl acetate solvent extraction, PFAS-targeted methanol solvent extraction, and 3-day desorption experiments in water. Results identified extractable and readily leachable polymer additives for use in the determination of release rate kinetics. PFAS compounds in extracts and leachates were quantified via HPLC-MS/MS and GC Orbitrap MS, while polymer additives were analyzed by HPLC with high resolution Orbitrap MS/MS. Compound annotations were based on matches to spectral libraries and a custom, curated database of known organic polymer additives and dyes. Based on library matching and/or computational mass spectrometry evidence, several known polymer additives were annotated, including Disperse Blue 373 and the chlorinated analog of Disperse Violet 93 in non-weathered PES fiber extracts, but not in the water leachate. 6:2 Fluorotelomer methacrylate (6:2FTMAC) was quantified at an average of $4.99e^4$ ng/g fiber in the non-weathered PES extract. A lower average of $1.06e^3$ ng/g was reported for the UV-weathered PES fiber extract. Results will be discussed in the context of release rate kinetics and UV exposure influence on polymer additive leachates.

04.05.13 Microplastic Abundance and Distribution in Organisms and Waters of the Non-Tidal River Thames, UK

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The abundance and effects of microplastic contamination have been well studied in the marine environment and the species which inhabit these systems. In comparison, the presence and effects of microplastics in freshwater systems have been less well studied. The River Thames (UK), the second largest river in the UK, has multiple anthropogenic stressors and pathways of potential microplastic contamination along its trajectory. This study aims to determine the abundance of microplastics in the non-tidal River Thames waters and the presence of microplastics in invertebrates and juvenile fish species that inhabit the river. Water samples, along with benthic dwelling invertebrate organisms and juvenile fish, were collected from 3 sites along the trajectory of the River Thames in May 2019. Sites were selected based on proximity to points of suspected microplastic contamination to the river. Samples were processed using physical separation and chemical digestions to extract and characterise microplastics found. A total of 6 surface water samples, 11 invertebrate species and 4 fish species were analysed. Preliminary results have identified the presence of microplastics in a range of taxa across different trophic levels of the River Thames ecosystem. Here, we discuss results to date which indicate widespread contamination. The potential ecological impacts for freshwater species in the non-tidal waters of the Thames will be discussed.

04.05.14 A Review of Laboratory Toxicity Tests of the Potential Effects of Plastic Leachate on Aquatic Organisms

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The release of chemicals from plastics is of concern due to the increasing global production of plastics and related increases in amounts of plastics waste in the environment. The potential effects of plastic additives or other weakly bound macromolecules that can leach into the aquatic environment are less well studied than physical effects of plastics on aquatic organisms. We conducted a review of the literature on the use of laboratory toxicity tests to assess the potential effects of plastic leachate on aquatic organisms. We identified, reviewed, and summarized 16 relevant laboratory studies conducted on a variety of plastic types. Crustaceans were the most common taxonomic group tested; others included microalgae, fish, bivalves, snails, and sea urchins. Acute mortality was the most common endpoint examined; others included algal growth and photosynthesis, reproductive and developmental effects, sublethal metabolic endpoints, and behavior. Exposure concentrations used to prepare leachate were reported in various ways, including mass of plastics per volume (e.g., mg/L), number of particles per volume, and as area of plastic per volume (e.g., cm^2 plastic/L). All studies except one reported adverse toxic effects, with mass-based 48-h LC-50 (or EC-50) ranging from 5 g/L to > 250 g/L. Comparison of the results of laboratory tests to field conditions is also complicated by differences in reported exposure metrics. For example, concentrations of microplastics (MP) in field-collected surface water samples are often reported as the number of particles per volume of water (e.g., number/L), the number of particles collected per surface area of water sampled by a towed net (e.g., number/m^2), or less often as the mass of particles per volume of water (e.g., mg/L). Concentrations of plastics used to prepare leachates in these laboratory studies far exceeded mass-based concentrations of MP in field collected samples as reported in the literature, which contributes to the uncertainty in our understanding of whether the effects of plastic leachate observed in the laboratory are occurring or will occur in the aquatic environment.

04.05.15 Determination of Microplastic Distribution and Uptake Using Cage Deployed Juvenile Chinook Salmon

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Fishes are an important part of food webs in aquatic ecosystems and are known to take up Microplastics (MPs). MPs have several potential impacts on fish including effects on food intake, and as a vector for other contaminants (e.g., pesticides) and microbiota. However, environmentally relevant studies monitoring MPs in fishes are rare as exposure and analysis are challenging and differ from case to case. Cage deployment studies can provide insight into the uptake and accumulation of MPs in fish – measured by the presence of MPs in tissues and feces following exposure in the field. Such studies can provide information about the differences between site conditions, variation between individuals or cages, and potential health effects on the organism, all of which are poorly understood for fish species.From March to May we deployed fall-run juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) for two weeks in cages at various locations in the Sacramento and Feather Rivers, as well as in the Sacramento-San Joaquin River-Delta in California. We sampled gills, livers, and gut content prior to deployment, at 7 days, and at 14 days to investigate the presence and characteristics of potentially ingested MPs. MP characterization was performed using Raman Microspectroscopy. Differences between tissues, individuals, cages, river site conditions, and the time points of deployments will be evaluated to assess the extent and potential for MP uptake. This research will provide methodological insights into the variability of MP uptake under realistic field exposure scenarios. It will also allow for the first characterization of MPs in this system, the potential for bioaccumulation of MPs over time, uptake variability, and influences of environmental parameters (e.g., flow and temperature) on uptake rates in Chinook Salmon. Knowledge gained from this study will help to determine future research needs in an ecologically and economically important fish and provide a basis for risk-assessment that will aid Chinook Salmon conservation managers.

04.05.16 Assessing the Effects of a Mixture of Microplastics in Natural Freshwater Zooplankton Communities

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Microplastics (plastic particles < 5mm) are globally ubiquitous contaminants and their potential effects on freshwater ecosystems are a growing concern. There is currently no scientific consensus on the toxicity of microplastics in natural environments and more research is needed to inform policy and risk assessment around microplastic contamination in freshwaters. To assess the potential effects of microplastics in freshwater ecosystems, a novel large scale in-lake mesocosm experiment is currently being conducted at the International Institute for Sustainable Development Experiment Lakes Area (IISD-ELA) in northwestern Ontario, Canada. Microplastics were added to 10m diameter x 2m deep in-lake enclosures in an experimental lake containing a range of environmentally relevant concentrations using a regression design (0, 6, 24, 100, 414, 1710, 7071, 29,240 particles/L). We added a mixture of microplastic fragments of three polymer types (PE, PET, PS) containing common chemical additives and ranging in size from 10-750µm. The objective of my project is to assess how microplastics affect the abundance, biomass and species composition of the freshwater zooplankton communities. Zooplankton are an important link between primary producers and higher trophic level organisms such as fish. Microplastics have been shown to negatively affect zooplankton reproduction, growth and mortality in laboratory studies, but we do not yet understand how microplastics will impact natural zooplankton communities, effects of which could have cascading impacts for freshwater ecosystems. The experiment will run for 10 weeks starting in June 2021 and results to date will be presented here.

04.05.17 Uptake and Elimination of Microplastics and Tire Wear Particles in the Eastern Oyster (Crassostrea virginica): Implications for Lowering Exposures to Human Consumers

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Microplastics (MP) are ubiquitous in coastal waterways and have been documented in filter-feeding organisms such as the Eastern oyster (*Crasssostrea virginica*). In South Carolina, oysters serve as an important food source for a variety of wildlife and humans. Previous studies in our laboratory have demonstrated that oysters collected from state-managed commercial and recreational harvesting grounds contain an average of 20 MP (±14.2 SE) per individual of which 21.4% of the particles were suspected tire wear particles (TWP). This suggests that a single serving of oysters (one dozen) contains on average 240 MP, which is s 6x more MP than a typical meal. The objective of this research was to characterize oyster MP and TWP uptake and determine if a subsequent depuration period is an effective method to reduce contamination. Oysters (n=70) were collected from Folly River near Charleston, SC and held in the laboratory in natural seawater for a three-day acclimation period. Following acclimation, oysters were individually exposed to either purple polyethylene fibers, green nylon fragments, and micronized crumb rubber (as a surrogate for TWP) in 600 mL beakers at an estimated concentration of 5000 MP/L. MP were aged in seawater for three days prior to use. Water was changed daily, and oysters fed an algae supplement during the exposure. Oysters (n=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 natural seawater and sacrificed at 24, 48, 96 hours (n=10) to characterize MP depuration. Oysters were digested with 10% KOH at 60°C for 24 hours and filtered for MP analysis. After 96 hours of exposure to fibers, oysters contained an average of 12.9 (±2.7) fibers per individual. Given 24 hours of depuration, MP contamination was significantly reduced to 5.7 MP fibers per individual (paired t-test, p=0.01) and after a 96-hour depuration period MP fiber abundance was reduced to 3.5 MP fibers per individual, representing a 72% overall reduction. Uptake and elimination of fragments and crumb rubber will also be presented. Results so far suggest that a depuration period in MP-free seawater could be an effective measure at reducing human dietary exposure to MP from oysters, although additional research is necessary to determine if these results would apply in situ with environmentally-derived MP.

04.05.18 Microplastics Exacerbate Disease Virulence in a Commercially Important Salmonid Species

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Global distribution of microplastics and their potential for biological impacts is garnering increasing concern of scientists, resource managers and the public. Historically, infectious disease is one of the greatest threats to ecosystem health. The interactions of disease and microplastics have been postulated, but remain largely unexplored. We evaluated the extent to which microplastics mediate the virulence of an important marine disease (Infectious Hematopoietic Necrosis virus, IHNV) in a model salmonid species, rainbow trout (*Oncorhynchu mykiss).* Populations of *O. mykiss* (threatened in some geographic ranges) may be augmented for human consumption with hatchery and aquaculture farming across the U.S. North Pacific, Europe and Japan, where IHNV can be widely destructive. To evaluate the hypothesis that microplastics may modulate the virulence or incidence of infectious disease, we conducted a controlled experiment in which trout were chronically exposed to varying doses (0, 0.1, 1 and 10 mg L^{-1}) of microparticles over an 8-week period, with acute IHNV exposure at four weeks. Microplastics were selected to reflect relevant fishery polymers, and included a nylon fiber (~10 x 500 µm) used in nets, and polystyrene (~20 µm diameter) ground from expanded polystyrene foam used in floats. Marsh grass was dried and ground (~20 µm diameter) and included as a natural microparticle control. Microparticle exposures (and no-particle controls) were conducted with and without IHNV exposure, in triplicate tanks of 20 fish each. No significant mortality from the presence of microparticles alone was observed. Mortalities were higher when microparticles and IHNV were co-administered, than IHNV exposure alone. This mortality increased significantly for nylon fibers at the high and medium concentrations and polystyrene at medium concentration (not following a dose response); mortality was also higher with natural microparticle co-exposure, but not significantly so. The increase in relative disease virulence following coincident microplastic and virus exposure may have arisen from increased susceptibility to infection (e.g., enhanced viral entry and replication) or compromised host defenses. This illustrates the potential for microplastics to be an ecologically significant co-stressor to aquatic biota and fishery resources, warranting further investigation.

04.05.19 Optimization of Larval Bivalve Microplastic Exposure System

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Microplastic pollution is ubiquitous in marine environments. Because inputs of plastic pollution continue to increase, it is essential to understand the impact that this will have on commercially-important marine and estuarine species. Bivalve larvae have been shown to take up micro- and nano-sized plastics; however, exposures detailed in the literature often do not account for particle dynamics such as corona formation, agglomeration, or settlement. There is clearly a need to define and maintain suspended particle concentrations during exposure periods, while using culture systems that maximize the growth of the organism. Therefore, we designed experiments to optimize the components of our exposure system for both particle suspension, as well as larval mussel growth. We assessed the suspension and clumping of 2 µm ultra-pure latex beads in artificial sea water at 24 and 48 hours to determine the influence of flask shape (flat, baffled, dimpled, and custom), movement (rotating and still), and dispersant type (lignin sulfonate, methyl cellulose, and gum arabic). These results were paired with an assessment of the effects of antibiotics (chloramphenicol), flask shape, flask rotation, and dispersant type on mussel larvae growth and survival. Baffled flasks had significantly lower survival. Custom flasks had significantly lower retention of particles in suspension at 48 hours and dimpled flasks had significantly higher retention of particles in suspension 24 hours. Overall, rotated flasks consistently performed significantly better than still flasks for all endpoints measured. In suspension experiments, both the low and high concentrations of methyl cellulose maximized suspension and minimized clumping. A 48 hour acute toxicity assessment of methyl cellulose and lignin sulfonate for mussel larvae resulted in an LC_{50} of 11.25 ppm for the lignin sulfonate and showed that methyl cellulose was not toxic to the mussel larvae up to the maximum concentration tested (100 ppm). The combination of these experiments revealed that the optimum system for exposure of bivalve larvae to micro-sized plastics was in rotating, dimple-bottom flasks with additions of 2.5 ppm methyl cellulose and 2 ppm chloramphenicol in natural filtered sea water. This system gives greater assurance of the true experimental exposure of the organisms to microplastics, allowing us to confidently create dose-response curves in assessments of the effects to commercially important larval bivalves.

04.05.21 Quality Criteria for Microplastic Effect Studies in the Context of Risk Assessment: A Critical Review

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In the literature, there is widespread consensus that methods in plastic research need improvement. Current limitations in quality assurance and harmonization prevent progress in our understanding of what the true effects of microplastic in the environment are. Following the recent development of quality assessment methods for studies reporting concentrations in biota and water samples, we propose a method to assess the quality of microplastic effect studies. We reviewed 105 microplastic effect studies with aquatic biota, provided a systematic overview of their characteristics, developed 20 quality criteria in four main criteria categories (particle characterization, experimental design, applicability in risk assessment, and ecological relevance), propose a protocol for future effect studies with particles, and, finally, used all the information to define the weight of evidence with respect to demonstrated effect mechanisms. On average, studies scored 44.6% (range 20-77.5%) of the maximum score. No study scored positively on all criteria, reconfirming the urgent need for better quality assurance. Most urgent recommendations for improvement relate to avoiding and verifying background contamination, and to improving the environmental relevance of exposure conditions. In far too many instances, studies suggest and speculate mechanisms that are poorly supported by the design and reporting of data in the study. This represents a problem for decision-makers and needs to be minimized in future research. In their papers, authors frame 10 effects mechanisms as 'suggested', whereas 7 of them are framed as 'demonstrated'. When accounting for the quality of the studies according to our assessment, three of these mechanisms remained. These are inhibition of food assimilation and/or decreased nutritional value of food, internal physical damage and external physical damage. We recommend that risk assessment addresses these mechanisms with higher priority.

04.05.22 Per- and Polyfluorinated Alkyl Substances (PFAS) Levels in Some Polytetrafluoroethylene (PTFE) Microplastic Powders - Implications for the Circular Economy

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The idea of a "circular economy" has gained significant momentum over the last several years. The circular economy is a vision for sustainable waste management where waste streams are diverted back into manufacturing with the goal of preventing the discharge of waste in order to protect the environment. Polytetrafluoroethylene (PTFE) and PTFE microplastics are poised as excellent candidates for recycling/upcycling in a circular economy due to their chemical inertness in the environment and essential niche industrial applications (e.g. as high-performance lubricants). However, since toxic per- and polyfluorinated alkyl substances (PFAS) are used to manufacture PTFE, there is concern that PTFE microplastics could be a source of environmental PFAS. To test this hypothesis, a number of industrial-grade PTFE powders in circulation (particle diameters ≤ 5 µm) were analyzed for PFAS using a high-pressure high-temperature liquid extraction followed by LC/MS/MS analysis. All samples contained levels of PFAS between 25 and 20,000 ppb, with the same general pattern of PFAS types present. The extraction of PFAS using high-pressure accelerated solvent extraction (ASE) demonstrated higher recoveries than liquid methanol extraction. This work demonstrates that some PTFE powders in circulation contain high levels of PFAS. Although PTFE microplastic powders are promising candidates for recycling in a circular economy, endogenous PFAS levels should be assessed in the recycling process.

04.05.23 Acrylic Fabrics As a Source of Microplastics from Laundry: Impact of Washing and Drying Parameters

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Microplastics are ubiquitous and considered as emergent pollutants that has severe detrimental effects on human being and aquatic environments. Increasing uses of synthetic textiles are now a concerning issue from the environmental perspective, as these synthetic textiles are shedding microfibers during washing and drying of the fabrics and are considered as a potential source of microplastics. Moreover, these fibers can pass through the wastewater treatment plants and eventually end up in the oceans. Manmade fibers like acrylic, polyester, and nylon represents 60% of the worldwide consumption of textile fibers. Thus, this study investigated the releasing trend of microfibers from acrylic fabrics during washing and drying in a real scale environment under different washing conditions. Washing and drying effluent from the washing machine were filtered out which were further quantified with gravimetric analysis to determine the net weight of released microfibers. Filter papers were analyzed by scanning electron microscope to determine the dimensions of the released microfibers. Overall, the mean length and mean diameter of the released microfibers were found to be 2411±1500 µm and 18±4 µm, respectively, despite having different washing parameters such as washing time, drying time, washing cycles, water temperature, and use of detergent. Under different conditions, released microfibers were varying from 1.6 mg to 201 mg per kg of fabric and 1.2 mg to 20.9 mg per kg of fabric during washing and drying, respectively. The results showed that more microfibers would release during both washing and drying of fabrics for longer time (124.69 mg/kg for 60 mins compared to 36.77 mg/kg for 30 mins washing and 71.25 mg/kg for 60 mins compared to 47.35 mg/kg for 30 mins drying) due to higher mechanical stresses on the fabrics. Moreover, microfibers were released approximately two times higher when washed with 40□ of water than with 20□, from both washing and drying, as high heat of the water loosening the fiber from the fabrics. However, subsequent washing cycles showed decreasing patterns of microfiber releases during washing, approximately 47% less in 7th wash compared to 1st wash. Similarly, during drying the fabric, fiber loss decreased by approximately 81% in 7th wash compared to 1st wash. These data might help to understand the releasing pattern of microfibers which can help to improve the present systems to reduce the microplastic emissions from laundering.

04.05.24 Microplastics: Comprehensive Workflows to Assess Their Occurrence and Fate

R. Marfil-Vega, Shimadzu Scientific Instruments

Estimates calculated in 2019 suggest that mass of microplastics in aquatic habitat will surpass that of fish by 2050. This prediction has dramatically changed with the impact of the COVID-19 pandemic as the use of single-use plastic products have increased since early 2020. To better understand the occurrence, fate and effects of microplastics and other contaminants associated with them, such as sorbed contaminants and other chemicals present in the plastic materials, a battery of complimentary analytical methods is essential. In this work we will present the results from applying a comprehensive analytical monitoring strategy for characterizing plastics, including IR microscopy, pyrolysis-GCMS and MALDI-TOF MS, demonstrate the method performance and discuss the value each of the techniques provide.

04.05.26 Analysis of Microplastic Pollution on Three Texas State Park Beaches

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"It's only one ___________ (water bottle, plastic utensil, straw, etc.)" said 5,000 SETAC Members. A recently published two-year survey of marine debris spanning the Gulf of Mexico (GofM) from North Padre Island, Texas to Santa Rosa, Florida found that marine debris accumulation rates were ten times greater in Texas than similar coastlines of the north central GofM, with 69-95% of the marine debris consisting of plastics (Wessel et. al., 2019). The goal of this study was to conduct and initial assessment of plastic pollution on Texas state park beaches (Galveston Island, Mustang Island and Sea Rim). Collections took place during the months of June and July (summer) and from September to November (fall) of 2019 (TPWD Pe No: 2019-R4-01). At each beach, we laid out a 50 X 1 meter transect on the high tide line. We Characterized the samples using a dissecting microscope with a camera attachment and measured each piece of small debris using ImageJ. Microplastic material composition was analyzed using FTIR-ATR spectroscopy. I characterized the spectral distributions of 1,000 samples of microplastics. Microplastics were found at all three sample sites at all collection times. The presence of microplastics on state park beaches indicates that microplastic pollution cooccurs with sandy beach meiofauna and macrofauna.

04.06 Nanoplastics Part I: Fate, Transport, and Exposure

04.06.01 Quantifying Breakdown of Plastic Products into Microplastics Using New Abrasion Method and Weathering

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The increased use of plastics in consumer products has led to the need of how plastics breakdown during use and in the environment due to increased pollution. The breakdown of larger persistent plastics into smaller microplastics (MPs) (and even smaller nanoplastics) will help quantify plastic degeneration rates and environmental impact. There are significant knowledge gaps include systematic studies under controlled conditions to understand how much MPs are abraded under specific uses (such as chewing, sanding, or ocean-wear) and then how much weathering affects abrasion tendencies. In order to answer these questions, a custom abrasion machine (intellectual property filed and NIST SOP pending) was used to acquire data to plot the generation rate of MPs versus power input during the abrasion of 3D printed polymers. All plastics were then tested in a greenhouse environment with rain, humidity control, and simulated sunlight for six months and one year and then underwent the same characterization and abrasion in the same machine. The six polymers chosen were polylactic acid (PLA), polycarbonate (PC), thermoplastic polyurethane 85A (TPU), polyethylene terephthalate glycol (PETG), high-impact polystyrene (HIPS), and nylon. Results indicate that the order of abrasion (from the easiest to break down into microplastics to the most difficult) were HIPS, PETG, nylon, PC, PLA, and then TPU. Model regression curves were fit to the generation rate of MPs vs. power input curves and the results were y= 0.30x (HIPS), y=0.24x (PETG), y=0.17x (nylon), y=0.16x (PC), y=0.07x (PLA), and y=0.05x (TPU). It is hypothesized that weathered plastics will have higher abrasion rates (higher slope values) than their counterparts. These equations were used in multiple product-use and environmental models (for example Kolmogorov's 1941 theory for ocean turbulence) to predict their MP output. When MPs were collected, the morphology of MPs varied between types but the average size was around 100 micrometers and nylon abraded into fibers. This methodology can be applied to the testing of all plastic consumer products to correlate their breakdown in the environment using controlled settings, especially once NIST SOP has been published. The MP generation rates and power inputs can be applied to specific products to better understand how their MPs release into the environment and in turn inform MP fate, transport and exposure models on the input of MPs from plastic product use and waste.

04.06.02 Generation and Characterization of Environmentally-Representative Nanoplastic Particles

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While the pervasiveness of plastic pollution in the environment is well-established, little is known regarding the extent, distribution, and effects of the smallest size fraction, referred to as nanoplastics. These materials pose unique sample collection and analytical considerations that have prevented their detection in the environment, while a lack of environmentally relevant reference materials creates a barrier to conducting representative exposure and effect studies. To address these challenges, there is an urgent need to develop a relatively simple and robust method for generating nanoplastic particles representative of those in the environment. The aim of this work was to develop a "top-down" approach for producing environmentally representative plastic test materials throughout the microplastic (>1 µm to ≤5 mm) and nanoplastic (≤ 1 µm) size ranges. Weathered marine macroplastic samples encompassing a range of polymers (including LD-/HDPE, PP, PS, PET, and Nylon) and waste electronic and electrical equipment (WEEE, also known as "black plastics") were chosen as environmentally-representative feedstock materials. Commercial-grade polymers (including LD-/HDPE, PP, PS, PET, Nylon, PEST, and ABS) were selected to serve as control materials to help identify methodological artifacts. Each feedstock material was individually cryomilled, bath sonicated, and then vacuum-filtered (1 µm PTFE) to generate and separate the micro- and nanoplastic particles. A variety of analytical techniques were used to characterize the materials at the each processing stage, including Fourier-transformed infrared spectroscopy (FT-IR), pyrolysis gas chromatography mass spectrometry (py-GC-MS), scanning electron microscopy (SEM), dynamic light scattering (DLS), and nanoparticle tracking analysis (NTA). Overall, the approach was found to effectively generate both micro- and nanoplastic particles. Higher yields were observed with the environmentally-representative feedstock materials compared to the commercial-grade polymers. The physical characteristics of the microplastic materials were generally consistent across the polymer types, typically fragments or flakes between ≈1 – 100 µm in size with rough, heterogenous surface features. The nanoplastic materials displayed more uniform shape and size characteristics, typically fragments between ≈100 – 300 nm in size. Chemical characterization is currently underway. *Disclaimer: This abstract does not reflect U.S. EPA policy.*

04.06.03 Field-Flow Fractionation Coupled Online to Raman Microscopy for the Analysis of Nanoplastic Particles

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Obtaining information about the size and chemical identity of nanoplastic particles (< 1000 nm), particularly in complex environmental matrices, is still very time-consuming as it usually involves laborious sample preparation and analysis, e.g. multiple centrifugation steps followed by further offline analysis using electron microscopy techniques and Raman Microscopy. We present here a novel analytical approach for the comprehensive characterization of nanoplastic particles using Field-Flow Fractionation (FFF) hyphenated with Raman Microscopy. While FFF enables the fractionation of nanoplastic particles according to their size and/or density, the hyphenation with Raman Microscopy allows for their simultaneous and non-destructive chemical identification. Online coupling of FFF with Raman Microscopy was achieved using a custom-made flow cell. This setup was successfully applied to particulate systems of different chemical compositions in the nanometer-range. A further coupling of the FFF-Raman setup to UV and MALS (Multi Angle Light Scattering) detection provided data on concentration (UV) and particle size (MALS) of the different nanoplastic components separated by FFF, allowing a comprehensive characterization in less than one hour per analysis.

04.06.04 Nanoplastic Particle Detection Through the Use of Surface Enhanced Raman Spectroscopy (SERS) Substrates Composed of Various Types of Gold Nanoparticles

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To date relatively few methods exist for the reliable detection of nanoplastic particles released into food products or the environment; particularly when the plastic particle concentrations are on the order of micro- or nanograms. This work aims to address this knowledge gap in the field through improving the scattering signal of sub-micro- and nanoplastic particles obtained during Raman spectroscopy measurements. To do so, surface-enhanced Raman scattering spectroscopy (SERS) substrates composed of gold nanoparticles (AuNPs) with various shapes and sizes were utilized. As a proof of concept, the substrates were used to analyze polystyrene (PS) sub-microparticles (161 nm) and PS nanoparticles (33 nm). The limits of detection (LODs) and analytical enhancement factors (AEFs) that could be achieved for the plastic particles were highly dependent on the SERS substrate utilized; with both the size and the shape of the gold nanoparticles the substrates are composed of impacting the final results obtained.

04.06.05 Geospatial Model to Estimate Microplastics Transport in the U.S. From Entering Waterways From Wastewater Systems and Land Applied Biosolids

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There is a need for exposure models to simulate the pathways and transport of such particles in waterways especially with increasing public awareness about the presence of microplastics in the environment. Microplastics may enter the environment from various sources and in many forms. One source includes personal care products containing plastic particles being washed down residential drains and entering municipal wastewater treatment plants (WWTPs). A large portion of these plastic particles are removed from the water phase during the treatment process, and generally end up in the solids (i.e., sludge). Sludge disposal varies by country, region and locality, including landfill, incinerator, compost, or as land-applied biosolids. There is potential for particles in biosolid applications to reach aquatic systems depending on application location and subsequent environmental conditions. This poster will present a broad-scale model designed to estimate emissions and model the fate of plastic particles exiting WWTPs into the terrestrial and aquatic environments in the United States. The model uses geospatial information on WWTPs, river hydrology and terrestrial transport potential. This regional/continental scale model is based on publicly available datasets and contained in a modular and transparent framework which is scalable and portable to multiple geographies. This presentation will demonstrate the utility of the model and how the resulting information about ultimate mass disposition (e.g., soil, freshwater, sediment, marine) and concentrations (surface water, sediment) can be used to help inform the discussion about prospectively assessing the presence and concentration of microplastics in the environment as emitted by WWTPs as effluent or transport from fields applied with biosolids.

04.06.06 Assessment of Drinking Water Treatment Processes in Nanoplastics Removal: Laboratory-scale, Pilot- Scale and Modeling Studies

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Microplastics have been detected in both potable water sources and tap water, leading to questions about the efficacy of current water treatment practices to remove these particles. Due to analytical difficulties, the detection of nanoplastics (NPs) both in the environment and in potable water is still challenging, however, it is hypothesized that water resources already contain NPs as degradation products of larger plastic items. The aim of this study was to investigate the behavior of NPs during ozonation and to assess their removal efficiency during sand and activated carbon filtration. Using metal doped NPs allowed us to quantify these particles in the treated water and in the filter media, where they are possibly retained. We measured breakthrough curves of NPs through sand and activated carbon filled columns and pilot-scale equivalents under different conditions to understand the processes which impact NPs retention. The MNMs software (Micro- and Nano-particles transport, filtration and clogging Model Suite) was used to model the breakthrough curves obtained from the pilot-scale DWTP to derive the hydrodynamic parameters of the filtration systems. These parameters were then used to simulate the behaviour of NPs in full-scale DWTP and estimate their removal efficiencies.Our results showed that at ozone doses typically applied in DWTPs, NPs remained mostly unaltered without influencing the NP transport in the subsequent filtration systems. In laboratory-scale column experiments, the filter length and flow rate impacted NPs retention in the filter media (sand and activated carbon). We assessed both pristine and aged sand and activated carbon, where the presence of a biofilm, which naturally occurs on the filter media, further reduced the concentration of NPs in treated water. In agreement with laboratory studies, results from the pilot-scale DWTP showed higher retention of NPs in the sand filtration opposed to activated carbon, in part due to the increased biofilm layer (Schmutzdecke). Both experimental and modeling results indicate a high capability of the filtration units in DWTPs to remove NPs from the source water with an overall log-removal of 3.6. The results from this study provide insights on the performance of current drinking water treatment technologies and can thus be used to assess the likelihood of NPs passing the DWTP and ending up in drinking water. However, further improved analytical methods need to be developed to measure nanoplastics removal through the entire DWTP in a full-scale operational plant.

04.06.07 Uptake, Accumulation and Depuration of Metal-Doped Nanoplastics in Oysters

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There is a considerable number of studies assessing micro and nanoplastics effects in bivalves, but not many on their uptake and accumulation. The main reason for this is the lack of analytical methods to track nanoplastics in the tissues and assess the potential bioaccumulated concentration. Doping nanoplastics with a scarce metal (Palladium-Pd NPs) can aid to track particles in complex environmental matrices, by the use of highly sensitive standard methods for metals as Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), allowing the use of more environmentally relevant concentrations for in vivo experiments. Allied with Transmission Electron Microscopy supported by Energy Dispersive X-ray Spectroscopy (TEM-EDX) it enables a detailed view of Pd NPs localization in tissues. Oysters *Crassostrea gigas* were exposed to two treatments of Pd NPs (size: 160-190 nn): SMOOTH and RASPBERRY at a concentration of 4 µg Pd L^{-1} (0.49% Pd per nanoplastic) for 6 days followed by 30 days of depuration. The SMOOTH and RASPBERRY treatments consist of particles with a smooth and an irregular surface morphology, respectively. Oysters were sampled at the start of the experiment (T0h) and after 3h, 8h, 24h, 3 and 6 days of uptake and 24h, 6, 12, 19 and 30 days of depuration. Different segments of the oysters were sampled at each day: haemolymph, gills, digestive gland, "rest" (remaining tissues) and faeces. The specific aims of this study were to (1) assess the concentration of Pd NPs in the different tissues of the oyster following exposure (2) understand the retention time of the particles after a long depuration period and (3) determine the accumulation and elimination rates. Thus far, the TEM-EDX analysis revealed the presence of the Pd NPs in the haemolymph after 24H of uptake. On day 6 of uptake, the RASPBERRY-Pd NPs were detected in the digestive gland. The SMOOTH ICP-MS data shows an increase of the Pd concentration with time, reaching a maximum on day 6 of uptake and a sudden decrease at the start of the depuration. The digestive gland was the tissue with the biggest concentration of the particles, followed by the gills and the "rest". Faeces had the biggest Pd content of all compartments with a maximum on day 3 of uptake. The concentration found in the haemolymph was < LOQ at all times. Our results demonstrate that most of the nanoplastics bioaccumulate in the digestive gland of the oyster and the concentration rapidly decreases when the dosing of Pd NPs stops.

04.06.08 Summary Report on the Nanoplastic Research Workshop Held by the European Commission Joint Research Centre and the U.S. National Institute of Standards and Technology

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Gorham, National Institute of Standards and Technology / CSTL; A. Held, European Commission - Joint Research Centre / Institute for Reference Materials and Measurements Standards for Innovation and Sustainable Development; Y. Lee, A. Madison, National Institute of Standards and Technology; D. Mehn, European Commission-Joint Research Centre; E. Petersen, National Institute of Standards and Technology / Biosystems and Biomaterials Division; J. Pettibone, D. Ripple, National Institute of Standards and Technology; B. Sokull-Kluettgen, European. Commission - Joint Research Centre / Nanobiosciences; S. Stavis, National Institute of Standards and Technology; L. Sung, National Institute of Standards and Technology / Engineering Laboratory; C. Zangmeister, National Institute of Standards and Technology

Nanoplastics, defined here as plastic particles smaller than 1 µm, are of growing concern for public health and environmental protection. However, their small size and organic composition make detecting them in complex environmental matrices nearly impossible with today's analytical chemistry testing technology. Without being able to accurately measure their presence or concentration, many fundamental questions remain a mystery. Are they present in the environment or in our food? Can they accumulate in (human) tissues? What is the exposure level? What contribution do nanoplastics make to the mass balance of plastic pollution in the ocean? How quickly do they degrade to the oligomer or monomer level? And finally: do they pose a risk to human health or ecosystems? Regulatory activities reducing plastic pollution are ongoing, as well as banning intentionally manufactured micro(nano)plastics added to consumer products in Europe (REACH), the U.S. (California State) and other countries, while analytical methods to detect and quantify nanoplastics in products or in the environment are not available or only at the development stage. The European Commission's Joint Research Centre (JRC) and the U.S. National Institute of Standards and Technology (NIST) organized a closed-door, virtual workshop to exchange knowledge, capabilities, and ideas on nanoplastic research with the goal to identify optimal advancement of methods in a collaborative manner. Presentations on the following topics were given by scientists from both organizations followed by 1-hour virtual discussion sessions: 1) toxicity and biological effects, 2) degradation, fragmentation and weathering, 3) separation, detection, and quantification methods, and 4) standard and test material production. The often analogous, and occasionally complementary capabilities of each organization emphasized the state-of-the-science. Together these capabilities can be leveraged to produce urgently needed materials, methods and approaches. This presentation will summarize both those broad capabilities and the state-of-the-science. We conclude with thoughts on productive directions for extending analytical capabilities and available standards.

04.07 - New and Existing Chemical Contaminants: Fate and Impacts in Changing Arctic and Antarctic Environments

04.07.01 Model-Based Assessment of the Long-Range Transport Potential of Chlorinated Paraffins

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Chlorinated paraffins (CPs) are complex mixtures with thousands of congeners and isomers. The potential environmental risk posed by CPs is of considerable concern due to large and increasing production volumes. While the complexity of CPs provides challenges to the assessment of their environmental fate and behaviour, the combination of environmental contaminant fate models with quantitative structure–property relationships (QSPR) holds promise as a means to assess their environmental fate. Of particular interests are differences in the behaviour of different CP constituents and the changes in the relative composition of CP mixtures that might be expected to occur as those mixtures are undergoing environmental transport and transformation processes, e.g. in the atmosphere. Here we estimate for 28,873 CP congeners (i) the partitioning properties K_{OA}, K_{OW} and K_{AW} with poly-parameter linear free energy relationships (ppLFERs) using QSPR-predicted solute descriptors, (ii) the rates of degradation in different environmental media using QSPRs implemented in EPISuite, and (iii) transport and target-oriented indicators or long-range transport potential (LRTP) using multimedia fate models such as the OECD POV & LRTP Screening Tool, ELPOS, TapL 3, Globo-POP and the Nested Exposure Model (NEM). The predicted persistence and LRTP of CPs are highly related to their structures. Using generic scenarios, the LRTP metrics are predicted to increase with both the number of chlorines and carbons, with a stronger effect of the former. Atmospheric measurements of the relative abundance of CP congener groups in regions with variable source proximity and remoteness are limited, but the available evidence does not corroborate the model predictions. Many of the CPs, especially those with long carbon chains and a high degree of chlorination, are likely to be particle-bound in the atmosphere and therefore have predicted LRTP indicators that are simply equal to that of the particles. Many of the models used for the assessment of LRTP of organic contaminants are ill-equipped to predict the atmospheric transport behaviour of particle-bound substances. More realistic predictions of the LRTP of CPs would therefore require (i) quantitative knowledge of the reactivity of CPs bound to particles and (ii) an improved understanding of the LRTP of the particles to which CPs are sorbing. Also, more atmospheric measurements allowing for an assessment of the relative LRTP of CPs of different carbon chain length and degree of chlorination are urgently needed.

04.07.02 Microplastics Pollution of Soils and Intertidal Sediments at Fildes Bay, Maritime Antarctica

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The Antarctic Peninsula has been described as an area with high human pressure, mainly due to the high number of scientific stations and the consequent activity they carry out, which generates a constant use and disposal of plastics from the Antarctic coastal systems. In 2018 a total of 11 samples were collected in King George Island. A transect in soils from facilities until Fildes bay (S1-S5) was made. Also, a second transect was used for intertidal samples along shore (IS1-IS5) and a reference sample was extracted from Ardley island (IS6). The samples were refrigerated at 4°C and, then, grain size, organic matter, pH and counting analysis were determinated. The particles were analyzed by Fourier-transform infrared spectroscopy (FT-IR). Microplastic were found at all sites. Soils were dominated by fragments with 4-37 particles/50 ml sample, but only 1 fiber/50 ml sample. On the contrary, intertidal sediments reached 1-4 fiber/50 ml sample, and fragments were not registered. A relationship between fragments and organic matter and soils S1, S4 and S5 and, also, there is a relationship between fibers, grain size and intertidal sediments IS1 and IS2 was determined by principal component analysis. The results shown a difference between soils and intertidal sediments for fragments ($\chi2 = 8.919$; $p = 0.003$), mainly because the sites S5 and S1 reached higher abundances (37 and 11 fragments/50 ml sample, respectively). Grain size varied significantly among soils and intertidal sediments ($p =0.0006$), and shows a higher size in intertidal sediments (average= 706.94 µm). Organic matter differs significantly among soils and intertidal sediments ($\chi2= 7.534$; $p = 0.006$) with higher amounts in soils (average= 5.95%). A positive correlation between fragments and organic matter ($p=0.779$), and for fibers and grain size ($p=0.713$) were found. The fragments had a bright orange color whose composition was phenoxy resin. On the other hand, the fibers presented different colors, with a PET dominance in all the sites, but also the presence of cotton in the sites surrounding the Frei base effluent. To our knowledge this should be the first report of microplastics in Antarctic soils, and the potential source may be the coatings surfaces used in the local bases and the effluent discharges for the fibers observed in the intertidal sediments.Thanks ANID-PFCHA/Doctorado Nacional/2017-21170746. ANID – Millennium Science Initiative Program – ICN2019_015. INACH/Correos de Chile PR_01-18.

04.07.03 Quantification of Microplastics and Organic Contaminants in Antarctic Soils Across Different Landscapes

K. Carroll, Arizona State University / School of Life Sciences; B. Polidoro, B. Ball, Arizona State University / School of Mathematical & Natural Sciences

Over the past decade, microplastic research has dramatically increased in interest, but is still mostly focused on the aquatic environment. Although Antarctica is considered a pristine environment, microplastics have recently been detected in the ocean and in ice-cores, while the detection of microplastics in soil has largely been ignored. Here, we investigate the presence of microplastics and organic contaminants in soils across a spectrum of different Antarctic landscapes. Soil samples from six different Antarctic field sites were collected, three in close proximity to research stations, and three from sites rarely or never visited. The presence and quantities of microplastics in soils were identified using Nile Red and fluorescence microscopy, while gas chromatography - mass spectrometry was used to detect phthalates, polychlorinated biphenyls, and other organic contaminants present in soils or associated with microplastic particles. It was hypothesized that the presence, concentrations and types of plastics and organic contaminants in different field sites, representing different levels of anthropogenic activity, can provide information on the potential sources and transport mechanisms of these pollutants to and within Antarctica. Results can therefore be used to inform policies for mitigating both local and long-distant sources of microplastic and organic pollutants in Antarctica.

04.07.04 A Comparison of PCB Congener, Organochlorine Pesticide, and Industrial Compound Burdens in Firn and Ice Cores From Antarctica and Svalbard

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Firn and ice cores from glaciers and ice sheets at opposite polar sites, Site M, Dronning Maud Land, East Antarctica (Lat 75.00 S, Long 15.00 E), and Holtedahlfonna, Svalbard (Lat 79.13 N, Long 13.27 E), were analyzed for 81 polychlorinated biphenyl (PCB) congeners, 23 organochlorine pesticides (OCP) and 16 organohalogen (chlorine/bromine) industrial compounds – mostly chlorobenzenes (OHIC). Both sites are remote, at high elevations, and receive contaminant inputs from long-range atmospheric transport (LRAT). The goal was to measure the difference in contaminant burdens at the 2 sites and to measure high/low ratios. At Site M, contaminant deposition and resulting burden results from subduction from the stratosphere to the free troposphere and to high-elevation surfaces, while at Holtedahlfonna, the sources are in the free troposphere from northern Eurasia. The results show differences in the three groups and a large variance for some classes between sites. The PCB burden at Site M is 172 pg cm^{-2} while the Holtedahlfonna whole core amount is 658 pg cm^{-2}, resulting in a high/low ratio of nearly 4. Site M PCBs were found mostly in the pentaCB homologue, missing important tetraCB congeners including PCB-52 and PCB-66, while at Holtedahlfonna, most PCBs were among tetraCBs. The burden of OCPs as a group at Site M is 556 pg cm^{-2} and Holtedahlfonna is 24 300 pg cm^{-2} a a high/low ratio of 44, apparently from the greater use of OCPs in the northern hemisphere: 6 OCPs were also not detected at Site M. Most of the OCP difference between the two sites results from different burdens of α-HCH, γ-HCH, chlorpyrifos and dacthal (the latter 3 current-use pesticides during time periods covered by the cores) that had high/low ratios of 121, 229, 43, 241. The burden of all OHICs at Site M were 7 300 pg cm^{-2}, by far the greatest amount of the three compound classes, and a factor of 1.85 lower than Holtedahlfonna (13 500 pg cm^{-2}). The small high/low ratio between the OHIC values may result from the high production of chlorobenzenes, particularly the dichlorobenzenes, in USA and Europe since the early1920s: In 1979 alone the production of 1,2-dichlorobenzene in Europe was 29 000 MT. High production, combined with high volatility of OHICs likely resulted in high concentrations and rapid dispersal throughout the global atmosphere.

04.07.05 A Survey of Perfluoroalkyl Substances in Water and Zooplankton of Lake Melville, Northern Labrador, Canada

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Perfluoroalkyl acids are a type of perfluoroalkyl substances (PFAS) that are highly persistent synthetic compounds. These compounds are detected in aquatic and terrestrial ecosystems and have a global ubiquitous presence. Lake Melville is an oligotrophic semi-enclosed estuarine fjard located in northern Labrador (Canada). This subarctic region is undergoing many environmental perturbations including climate warming and hydroelectric power development. The hydroelectric power project is centered in Muskrat Falls and has been subject to scrutiny due to potential environmental impacts arising from mercury contamination caused by clear-cutting, flooding and reservoir creation. The environmental perturbations in Lake Melville may also have ramifications on other contaminants such as PFAS. The objectives of this study are to investigate concentrations and congener profiles of PFAS in water and zooplankton in Lake Melville across a freshwater-marine gradient . Here we report plankton-water bioaccumulation factors (BAF). In total 18 samples of water and 21 samples of invertebrates were analyzed for 17 PFAS using ultra-high performance liquid chromatography coupled with tandem mass spectrometry. PFAS are ubiquitous in Lake Melville water and zooplankton. The soluble short-chain PFAS congener, perfluorobutanoic acid (PFBA), was predominant in Lake Melville surface and subsurface water. PFAS was higher in the freshwater portion of the fjard, both horizontally along the freshwater-marine gradient and vertically in the stratified water column, likely due to riverine inputs. Concentrations decreased across the salinity gradient, due to dilution by marine water from Labrador Sea (which flows landward) and decreasing proximity to sources. The PFAS burden showed a similar gradient in lake zooplankton. Logarithmic water-biota bioaccumulation factors (BAF) for perfluorooctane sulfonate (PFOS) in zooplankton was positively correlated with $\delta^{13}C$, illustrating that bioaccumulation of PFOS is partly driven by the carbon or energy flow at the base of the ringed seal food web of this estuary. Other factor such as different dominant phytoplankton and zooplankton communities, and seasonality that determines phytoplankton status and extent of sea ice, also affect bioaccumulation of PFAS in these organisms. This study is the first to investigate PFAS in Lake Melville water and zooplankton.

04.07.06 Increasing Trends of Flame Retardants and Perfluoro-Alkyl Substances in Char (Salvelinus Alpinus) in Remote Arctic Lakes; Climate Change Influence?

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Landlocked Arctic char are the only top predators in most high Arctic lakes and, therefore, can serve as a sentinel species for changes in atmospheric inputs of persistent organic pollutants. Atmospheric measurements and studies of ice cap snow/firn and ice cores published over the past 10 years have shown continuing and, in some cases, increasing, inputs of polybromo-diphenyl ethers (PBDEs) and poly/perfluoroalkyl substances (PFAS) to the high Arctic. We were interested to see if these trends were reflected in the landlocked char as well. We determined PBDEs (13 Br3-Br7 congeners) and PFAS (C6-C14-perfluoro carboxylates (ΣPFCAs) and perfluorooctane sulfonate, PFOS) in muscle of Arctic char and compared results to published global emission estimates. Data was available for 4 lakes, spanning 12 to 19 sampling years from the early-1990s to 2019, with sample sizes ranging from 3 to 10 fish per year. Σ_{13}PBDEs significantly increased in 3 of 4 study lakes (Char, Resolute, and Hazen) from the 1990s to 2018, ranging from 6.6 to 9.1%/y. In a 4th lake (Amituk), Σ_{13}PBDEs were highest in samples from 2000 to 2004 and then declined in the mid-2000s before rising over the period 2006 to 2018. The increase of PBDEs contrasts with phase out and bans of PBDEs in the mid-2000s and modelled predictions of declining global emissions. PFOS in char muscle appeared to increase over the period 2010-2019 in two very remote lakes (Amituk and Hazen) although the trends (6-7%/y) were not statistically significant. However, from 2015 to 2019 the increase in PFOS concentrations in Amituk was more rapid (40%/y). Similarly ΣC_7-C_{14}-PFCAs also increased significantly in Amituk and Hazen lakes over the period 2012-2019 (12-20% /y). The increasing trends of ΣPFCAs and PFOS in char are surprising given the phase-out of perfluorooctanoic acid and related PFAS by 2014 as well as PFOS in the early 2000s and modelled predictions of declines in volatile PFAS-related precursors that would undergo long-range transport. Nevertheless, the general trends of ΣPFCAs and PFOS in char are in agreement with published snow/ice and air measurements from the mid-2000s to 2015. Climate related factors could be important. A huge increase of glacial meltwater inputs to Lake Hazen, occurred from the mid-2000s to 2013. In the case of Char and Resolute Lakes, which are near an airport, increases in PBDEs in could reflect remobilization of local contaminants due to warming soils and more intense summer rains.

04.07.07 Identifying Pathways of Methylmercury Through Marine Food Webs of the Aleutian Islands Using Compound Specific Stable Isotope Analyses of Amino Acids

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The waters surrounding the Aleutian Islands, Alaska support important fisheries for humans and top marine predators, including Steller sea lions (*Eumetopias jubatus*; SSL). The western Distinct Population Segment of SSL is listed as endangered under the US Endangered Species Act due to steep population declines during the 1970s and 1980s with subsequent and continuing failure to recover. While the reasons for this remain unknown, monomethylmercury (methylmercury or MMHg) has been identified as a possible stressor affecting SSL. This is particularly relevant for SSL from the central (CAI) and western (WAI) Aleutian Islands, where total mercury concentrations ([THg]) in SSL tissues and their prey are higher than other areas of coastal Alaska. A high-resolution understanding of trophic relationships is essential for deciphering the food web pathways that deliver MMHg to upper trophic level organisms in these regions. We are using compound specific stable isotope analyses (CSIA) of carbon and nitrogen (expressed as δ^{13}C and δ^{15}N values) of amino acids (AAs) in muscle of archived fishes and cephalopods from habitats bordering the CAI and WAI regions (n= 30 samples/region from 9 species) to infer trophic positions and routes of primary production. δ^{13}C values of essential AAs will chemically fingerprint primary production sources while δ^{15}N values of AAs estimate trophic position. This relatively new approach addresses uncertainties commonly associated with bulk stable isotope analyses. To date, we have analyzed δ^{13}C and δ^{15}N values of AAs from 60 yellow Irish lord (*Hemilepidotus jordani*), a benthic fish found throughout Alaskan waters. Results indicate δ^{15}N values of the AA phenylalanine, an indicator of the baseline of a food web, varied significantly across sampling sites. We also noted a positive correlation between trophic positions and [THg] of individuals sampled from the region west, but not east, of Amchitka Pass, a discrete ecological divide in the Bering Sea. Based on the carbon isotope fingerprinting, yellow Irish lord obtained most of their essential AAs from micro- or macroalgae regardless of sampling location. Future analyses with other species will determine if these patterns are consistent among species and between regions. Our novel coupling of these isotopic approaches to analyze relevant diet items of SSL is providing a more nuanced perspective of the pathways of MMHg transfer in these food webs.

04.07.08 Seabird-Mediated Transport of Organohalogen Compounds to Remote Sites

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In the present study, the role of seabirds for the transport of organochlorine compounds to very remote sites, e.g. western Greenland, is evaluated. Three lakes were selected for this purpose, one, NOW5, with a little auk (*Alle alle*) bird colony and the other two, NOW14 and Q5, devoid of these seabirds. NOW14 has a historic albeit inconsistent human presence record, and Q5 is away from either bird or human presence. Samples were collected from the freshwater lakes on the coast of NOW polynya in the summers of 2015, NOW5D and NOW14, and 2016, Q5. The cores were sliced and stored frozen until analysis. The sediments were extracted with ethyl acetate/cyclohexane (5:2, v/v) by vortex mixing and sonication. The total extract was then concentrated and activated copper was added to eliminate sulphur. Clean-up was performed using Florisil cartridges. The eluted extracts were analyzed by gas chromatography (GC) with electron capture detection and GC coupled to negative ion chemical ionization mass spectrometry. The waters of NOW5 had a strong acidic pH, 3.4, whereas the pH values of NOW14 and Q5 were close to 8. This difference can be attributed to the influence of *A. ale* guano depositions. Other differences between the waters of NOW5D lake and the others were related to the fertilizing guano effects. Thus, NOW5D showed high chlorophyll concentrations (74 µg/L vs. 1.6-3.4 µg/L, respectively), higher content of total phosphorous (0.34 mg/L vs. 0.007-0.01 mg/L, respectively) and total nitrogen (3.75 mg/L vs. 0.21-0.75 mg/L, respectively). These differences reflected that the waters of NOW5D received more nutrients than in the other two lakes. The concentrations of all organohalogen compounds were much higher in the lake under the influence of *A. ale* than in the other lakes showing the strong influence of these seabirds in the transport and deposition of these hydrophobic compounds to remote sites. However, not all compounds showed the same relative increases. The most volatile, hexachlorobenzene and the hexachlorocyclohexanes, were about 20 time higher in NOW5 whereas the enrichment of the chlordanes, PBDEs and PCBs was between 4 and 6 times and DDTs three times. These differences evidence a selective effect in the seabird accumulation and transport of organohalogen to remote sites. Seabirds play a significant role in the tranport of oranohalogen compounds to remote sites but their effect is selective depending on the chemical composition of these pollutants.

04.07.09 Variation in Sea Ice Extent, Prey, and Habitat Use Drive Perfluoroalkyl Acids and Hg Trends in an Arctic Seabird, the Thick-Billed Murre

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Arctic marine ecosystems are undergoing rapid change in connection with climate change. Arctic top predators such as seabirds are indicator species of marine ecosystem health, and are being impacted by shifts in multiple stressors, including changes in prey availability, sea ice extent, and contaminant concentrations. From 2016 to 2018, we examined total mercury (THg) concentrations and per- and poly-fluoroalkyl substances (PFAS) in blood samples of thick-billed murres (*Uria lomvia*) at Coats Island, Nunavut, Canada, and in relation to inferred diet, foraging habitat, and sea ice extent. Twenty-two PFAS (18 perfluoroalkyl acids (PFAAs) plus 4 precursors (FBSA, FOSA, N-MeFOSA and N-EtFOSA) were analyzed in the 2016 samples whereas only 18 PFAAs were determined in the 2017 and 2018 collected samples. Regardless, the most frequently detected and highest levels in the blood were perfluorooctane sulfonate (PFOS) and C_9-C_{13} perfluorinated carboxylic acids (PFCAs). Total Hg concentrations were highest in 2018, a late-ice year, relative to 2016 & 2017 (early ice), whereas PFAS and several congeners were lowest in 2018. Total Hg concentrations increased with carbon ($\delta^{13}C$) & nitrogen ($\delta^{15}N$) stable isotope values, but decreased with sulphur ($\delta^{34}S$), suggesting higher THg concentrations were associated with higher trophic and benthic prey. On the other hand, ∑PFAS and several congeners decreased with $\delta^{13}C$ values, but increased with $\delta^{34}S$, suggesting higher PFAS concentrations from offshore pelagic prey. Similar to the trends in THg and PFAS, $\delta^{13}C$ values were highest in 2018 whereas $\delta^{34}S$ values were lowest in 2018, with no difference between 2016 and 2017. There was also no overlap in the niche region size of murres from 2018 with previous years, suggesting murres were feeding on different prey sources. There was also less overlap among the foraging areas of murres in 2018 with previous years. We believe that inter-annual variation in sea ice extent affected the prey and habitat use in murres, resulting in differences in contaminant concentrations. We conclude that THg and PFAS in murres are from different sources, and variation in sea ice extent may lead to changes in contaminant transfer to top predators and Arctic marine ecosystems.

04.07.11 Retrospective Assessment of Plastic Additives in Eggs from Seabirds Breeding at the Prince Leopold Island Migratory Bird Sanctuary From 1975 to 2019

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Arctic seabirds are known to ingest and accumulate microplastic debris. Microplastic pollution does not represent a single contaminant, but rather a “cocktail” of contaminants including physical pieces of plastics and a suite of plastic-associated chemicals (e.g., plasticizers, flame retardants, and stabilizers). Recent studies have identified various plastic stabilizers such as UV absorbents and industrial antioxidants in Arctic seabird eggs and livers. Given that many of these contaminants are known endocrine-disruptors, it is important to understand how these northern seabirds have been exposed to these plastic-derived contaminants over time. The objective of this project was to investigate the occurrence and temporal trends of plastic stabilizers including synthetic phenolic antioxidants (SPAs), secondary aromatic amines (Ar-SAs), benzotriazole UV stabilizers (BZT-UVs) and various UV filters (UVFs) in the archived egg samples from three seabird species (Thick-billed Murres (*Uria lomvia*), Northern Fulmars (*Fulmarus glacialis*; NOFU) and Black-legged Kittiwakes (*Rissa tridactyla*)) that have been collected from Prince Leopold Island in collaboration with the community of Resolute Bay and the Sulukvaut Area Co-Management Committee between 1975 and 2019. The preliminary results of five NOFU eggs collected in 1975 showed that the most frequently detected contaminants were 3,3,5-Trimethylcyclohexyl salicylate (HMS) (100%), 2-6-Di-tert-butyl-4-methylphenol (BHT) (80%), 2-(2H-benzotriazol-2-yl)-p-cresol (UVP) (60%), 2-(2H-benzotriazol-2-yl)-4,6-bis(1-methyl-1-phenylethyl)phenol (UV234) (60%) and Bis(4-(2,4,4-trimethylpenta-2-yl)phenyl)amine (C8C8) (60%). The median concentrations were 5.7 ng/g, 1.6 ng/g, 4.2 ng/g, 5.2 ng/g (wet weight basis) for HMS, BHT, UVP, UV234 and C8C8, respectively. The levels of C8C8 and UV234 in 1975 NOFU eggs were higher than the previously reported concentrations in 2013 NOFU eggs, indicating a possible decline of these contaminants in NOFU eggs. This research provides a basis for understanding the exposure risks of Arctic seabirds to various plastic stabilizers and more research is warranted to elucidate the toxicological risks these emerging contaminants may pose to wildlife in the Arctic.

04.07.12 Maternal Transfer of Legacy and Emerging Poly- and Perfluoroalkyl Substances to Eggs in an Arctic Seabird

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To enable embryo development, the avian egg receives numerous nutrients and substances from the female during its synthesis, though maternal transfer is also a major route of contaminants exposure to developing birds. In pre-laying black-legged kittiwakes females (n=14) from Svalbard and their eggs (n=25), we measured legacy and emerging poly- and perfluoroalkyl substances (PFAS) including fluorinated alternatives (Gen-X, ADONA and F-53B). We aimed at 1/ describing the contaminant levels and patterns in both females and eggs, and 2/ investigate the maternal transfer, i.e. the relationship between the females and their eggs for each compound. We found 7:3 fluorotelomer carboxylic acid - a precursor of long-chain carboxylates in 84% of the eggs, as well as ADONA in a single egg. These compounds were all below the detection limit in female plasma. We recommend additional effort on screening for emerging PFAS alternatives in free-ranging top-predators. Contamination of both females and eggs were dominated by linPFOS then PFUnA or PFTriA. Concentrations generally decreased with laying-sequence. There was a linear association between females and eggs for most of the PFAS. Analyses of PFAS ratios in females and eggs suggest that longest chain PFCA ($\geq C_{12}$) are preferentially transferred to the eggs.

04.07.13 Using Fatty Acid Signatures to Better Understand How Feeding Ecology Influences Contaminant Concentrations Among Ringed Seals at Four Sites in the Canadian Arctic

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While it is challenging to assess climate change-contaminant interactions, one possible approach, which is often used in climate change community ecology research, is “space-for-time” substitution. As applied to climate change-contaminant interaction studies, potential impacts of future climate-driven feeding changes on contaminant exposures in biota could be inferred by comparing current spatial differences in feeding patterns and their impacts on differential contaminant exposures among regions. In the present study, we aimed to understand how spatial variation in feeding ecology influences the blubber tissue concentrations of persistent organic pollutants and mercury in ringed seals (*Pusa hispida*) across two Canadian sub-Arctic sites (Nain at 56.5°N, Arviat at 61.1°N) and two Canadian Arctic sites (Sachs Harbour at 72.0 °N, Resolute Bay at 74.7 °N). Through the Northern Contaminants Program, blubber tissues were collected in the summer (Resolute Bay and Sachs Harbour) and fall (Arviat and Nain) of 2018 from ringed seals harvested by community subsistence hunters. Seal samples have been analyzed for polychlorinated biphenyls, organochlorine pesticides, toxaphenes, flame retardants; per-/polyfluoroalkyl substances and mercury. Here, major diet-derived fatty acids (16:3n6, 18:2n6, 18:4n3, 20:1n9, 20:1n7, 20:5n3, 22:1n11, 22:6n3) found in the seal blubber were used as tracers of feeding patterns. Principal component analysis demonstrated overlapping fatty acid signatures between ringed seals from Nain and Arviat and limited overlap between Nain and Resolute Bay, suggesting differences in feeding patterns between these sub-Arctic and Arctic sites, respectively. Differences in regional ecosystems and feeding ecology may explain these differences. The northward range shift of non-native fish species, which has already been documented within the Canadian sub-Arctic, could also contribute to the explanation. Nonetheless, the other Arctic site, Sachs Harbour, showed the largest ellipse of fatty acids, suggesting greater dietary diversity. Next, we will examine associations between these observed feeding patterns and the contaminant concentrations among sites to better understand how feeding differences influence spatial variation in contaminant concentrations among ringed seals. Results will further understanding of how climate change may influence contaminants in Arctic marine biota.

04.07.15 Historical Circumpolar Distribution of Perfluoroalkyl Acids, Precursors and Isomers in Polar Bears From Alaskan, Canadian, East Greenland and Svalbard Subpopulations

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Polar bears (*Ursus maritimus*) in the Arctic are contaminated with per-/poly-fluororalkyl substances (PFAS), and in particular with perfluoroalkyl acids (PFAAs). There has yet to be a comprehensive examination of the circumpolar distribution of bioaccumulative PFAS in polar bear subpopulations. The present international study carried out a historical examination of 22 PFAS, and the majority being perfluorinated sulfonic acids (PFSAs) and carboxylic acids (PFCAs), in liver samples of 139 individual polar bears from 11 of the 19 circumpolar management zones/subpopulations and collected over the years of 2005 to 2008. Twelve of the 22 PFAS were non-detectable in any sample, i.e. PFBS, PFDS, PFHxA, PFHpA, PFTeDA, PFPA, PHUEA, FOUEA, FDUEA, FHET, FOET and FDET. Based on the spatial distribution of PFAS, bears from East Greenland had significantly higher liver concentrations of PFOS (2500 ng/g) and ∑PFSAs (2600 ng/g) than all other subpopulations ($p < 0.05$). Bears from Southern Hudson Bay and Baffin Bay subpopulations also had higher PFOS concentrations of > 1000 ng/g compared to the remaining populations. ∑PFCA tended to be higher also for Southern Hudson Bay, Baffin Bay and East Greenland bears. PFOS isomeric patterns (linear, mono-branched and di-branched) in 43 of 139 polar bear samples from eight of the management zones showed linear PFOS and 6 mono-branched isomers in all analyzed samples with the linear PFOS accounting for > 90 % of the ∑PFOS-isomer concentrations for seven of the eight investigated management zones. The exception were bears from the Barents Sea in which proportion of linear PFOS (82 ± 3.2 %) was significantly lower than for any of the other bear subpopulations ($p < 0.0001$). Of the linear correlative relationships between PFAS concentrations (log transformed) and 14 individual biological factors of the polar bears, several dietary fatty acids, i.e. linoleic acid, α-linolenic acid, arachidonic acid, docosapentaenoic acid, cis-8,11,14-eicosatrienoic acid and cis-11,14-eicosadienoic acid, strongly suggested an underlying connection between PFSA accumulation and dietary differences among the bear subpopulations.

04.07.16 Variation in Tissue Concentrations of Legacy POPs in Four Marine TOP Predators in a Changing Arctic

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Polar bears and northward-redistributing killer whales show orders of magnitude differences in tissue concentrations of legacy persistent organic pollutants (POPs). Yet, comparisons across regions and different time frames may, at least in part, confound these reported interspecific differences. Other species, such as endemic narwhals and northward range-shifting pilot whales, have been too data deficient with respect to POP concentrations to be included in quantitative assessments. Here, we used opportunistically-collected samples from subsistence-harvests of four top predator species within the same broad region (southeast and East Greenland) and over a similar time period (2013-2020) to assess interspecific variation in blubber/adipose polychlorinated biphenyl (PCB) and organochlorine (OC) concentrations. We further determined fatty acid (FA) signatures, which are used as diet indicators, to evaluate the extent to which variation in POP concentrations were explained by differences in feeding habits. Very high mean concentrations of ΣPCBs, Σchlordanes (ΣCHLs), hexachlorobenzenes (ΣHCBs) and Σdichlorodiphentlytrichloroethanes (ΣDDTs) were found in killer whales (~40 to 70 mg kg^{-1} lipid weight) relative to polar bears (~0.1 to 5 mg kg^{-1} lipid weight). A PCA on FA signatures show that feeding patterns are most distinct between killer whales and polar bears, while narwhal and pilot whale are intermediate. Generalized linear models using PC scores of the FAs as explanatory variables for the POPs concentrations demonstrate that dietary differences explain a significant amount of the variation for multiple POP classes. This first quantitative comparison shows significant variation in POP concentrations among Arctic marine top predators and that feeding ecology may play a role in shaping this variation. Variation in POP concentrations between toothed whales versus polar bears is likely additionally related to phylogeny, with toothed whales having reduced metabolic ability to eliminate lipophilic persistent environmental- pollutants such as PCBs compared to carnivorous predators such as polar bears. Our findings on the influence of diet on POP concentrations have important implications for understanding of how climate change and associated food web changes may affect contaminant exposures among highly susceptible top predators in an ecosystem undergoing drastic changes.

04.08 Non-targeted Analysis: Approaches Toward Identification of Chemical Contaminants

04.08.01 Applications of Machine Learning to In Silico Structure Prediction for Non-Targeted Analysis with High-Resolution Mass Spectrometry

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Advances in non-targeted analysis have enabled the discovery of new chemicals that would previously remain undetected with traditional targeted techniques. Despite their great potential, current workflows can detect thousands of chemical features but fail to derive potential structures or confirm the majority of these compounds with MS/MS fragmentation spectra or with analytical standards. There is, thus, a need to develop new computational approaches for structure elucidation. The aim of our study was to develop a machine learning algorithm for *in silico* structure prediction and use it to propose potential chemical structures for compounds detected in blood samples. As input data for the algorithm, we used physicochemical properties that can be measured in the lab, such as equilibrium partition ratios between organic solvents and water (e.g., *K* octanol/water). As these properties are a function of a chemical's structural characteristics, we presume that they can also be used to back-calculate functional groups for a chemical given its physicochemical properties. Our hypothesis is that as these properties are sufficiently different across structural isomers when put together they can form a unique fingerprint for each isomer, which we describe as the "physicochemical spectrum". We developed and tested a preliminary version of the algorithm using the database Chemicals in Human Blood (n=927) from EPA's Chemicals Dashboard. The database is an aggregate of public resources and includes endogenous metabolites present in human blood. As input parameters, we used the *K* solvent/water for carbon disulfide, chlorobenzene, cyclohexane, dichloromethane, fluorobenzene, diethyl ether, ethyl acetate and 1-octanol. The algorithm was then trained to predict functional groups, such as RNH2, ROH, ROR, RCOOH, carbon rings, among several others. The algorithm was evaluated using a 5-fold shuffle-split cross-validation and a y-randomization. The developed algorithm showed promise in predicting functional groups, such as cyclohexane rings with reasonable accuracy (R^2 for the training set: 0.92, R^2 for the testing set: 0.73). The good agreement between the R^2 of the training set and that of the testing set indicates that the algorithm has the potential of making reasonable predictions outside its calibration domain. Our future studies will focus on leveraging larger databases and reevaluating the predictive power of the algorithm with increasing structural variability.

04.08.02 FluoroMatch: Processing Non-Targeted Mass Spectrometry Data for Per- and Polyfluoroalkyl Substances

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Per- and polyfluoroalkyl substances (PFAS) are distributed globally in products such as food wrappers, sticky notes, and carpets. PFAS last in the body for a long time, and some are highly toxic. PFOA and PFOS, two of the most studied PFAS, have been linked to certain cancers and other illnesses, and they impact various biological processes including reproduction and the immune response. There are nearly ten thousand PFAS for which the health impacts have not yet been studied, and there are likely still thousands of undiscovered PFAS. Although the structures can vary dramatically, the vast majority of PFAS have some kind of carbon-fluorine chain (e.g., $[CF_2]_n$ and $[CF_2O]_n$) which allows for an automated non-targeted approach. FluoroMatch is the first software to process non-targeted mass spectrometry data for PFAS. FluoroMatch provides six types of evidence that help build annotation confidence, scoring each identification from A+ to E. Identifications based on class-based MS/MS fragmentation rules from standards score in the A-range (confident), while any other annotations with MS/MS evidence score in the B-range (tentative). FluoroMatch also pulls out hits that fall within a homologous series based on Kendrick mass defect. Thus, any identification that falls in a series of CF2 units (or other common PFAS series) with a confident or tentative annotation scores in the C-range, and any otherwise unidentified annotations that lie in series score in the D-range. These B, C and D-range annotations are by no means confident, but they illuminate the sheer volume of possible PFAS and provide starting points for the discovery of new compounds. In leachate data, there were ~25 confident and ~50 tentative identifications, but there were ~170 hits in series with those identifications and a staggering 5000+ other hits in series. It is essential to determine which compounds have contaminated the environment and which are still used in consumer products in order to identify and regulate the most concerning compounds and to better educate on PFAS health effects. FluoroMatch is a software which automates non-targeted PFAS data-processing and assists researchers in working toward this goal.

04.08.03 Using a Chemical Derivatization and High Resolution Mass Spectrometry Workflow to Identify Microcystins in Two Drinking Water Sources and Recreational Waters

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Some of the most hazardous chemicals are natural toxins such the cyanotoxin, microcystin (MC). Microcystin is a cyclic heptapeptide hepatotoxin produced by several species of cyanobacteria. Anthropogenic actions have increased the number and intensity of freshwater toxic cyanobacteria blooms increasing the exposure risk through drinking water and recreational waters. USEPA has released a drinking water health advisory at 0.3 ppb MC for pre-school aged children and a recreational health advisory at 8.0 ppb MC. Based on current structural and statistical calculations, thousands of MCs can exist; yet, to date, only 270 MCs were identified and only 12 commercial MC standards are available. Standard mass spectrometry workflows for known and unknown MCs need to be developed and validated for basic and applied cyanobacterial bloom research to advance. Our investigation focuses on two drinking water source and a recreational water with total MC concentration by ELISA that were much higher concentrations reported by EPA method 544, a liquid chromatography tandem mass spectrometry method. The two drinking water sites were Oser and Bischoff Reservoirs, IN, and Grand Lake Saint Mary, OH. The recreational water site was in Sandusky Bay, OH. During sampling, the dominant cyanobacterium found at each of these water was Planktothrix agardhii. Our goal was to identify and quantify the MCs in these samples using chemical derivatization and mass spectrometry. We used thiol-ene chemistry to determine the amino acid in position 7, an online concentration liquid chromatography tandem mass spectrometry (LC–MS/MS) method, and high resolution mass spectrometry (HRMS) to discover untargeted MCs. In each of the samples, we discovered two untargeted MCs. The Oser and Bischoff Reservoirs had two MCs that have been tentatively identified as [D-Asp3, Dhb7]-MC-LR and [D-Asp3, Dhb7]-MC-HtyR. Sandusky Bay had two MCs that have been identified as isomers standards [D-Asp3, Dhb7]-MC-RR and MC-YR. These have been tentatively identified as [D-Asp3]-MC-RR and [D-Asp3]-MC-HtyR. Similar, Grand Lake St. Marys had two MCs have been tentatively identified as [D-Asp3]-MCRR and [D-Asp3]-HtyR. This is the first reported incidence of Dhb MCs in the United States. Furthermore, it was discovered that the commercially available [D-Asp3]-MC-RR standard was [D-Asp3, Dhb7]-MC-RR. In conclusion, our research suggests that current commercially available MC standards do not represent the prevalent MCs in several US waterbodies.

04.08.04 Per- and Polyfluoroalkyl Substances in Canadian Plant Fibre Based Fast Food Contact Materials

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Per- and polyfluoroalkyl substances (PFAS) have been intentionally added to food-contact materials (FCMs) for decades to confer grease and water repellency with high chemical and thermal stability. PFAS are persistent and mobile; some are bioaccumulative and toxic. As such, their use in FCMs is of concern in terms of human and environmental exposure throughout their life cycle. While the US FDA has a publicly available list of PFAS authorized for use in FCMs, Canada has a list of PFAS that have received a "Letter of No Objection" from Health Canada, but this list is not publicly available. In response to concerns, the food packaging industry has replaced the use of PFOS, PFOA and specific long-chain PFAS in favor of other PFAS where the latter undergo minimal regulatory scrutiny. We used a suite of analytical techniques to create a comprehensive picture of PFAS in a selection of Canadian plant fibre based fast food FCMs. These were chosen based on elevated levels of total F obtained using Particle-Induced Gamma Ray Emission (PIGE) spectroscopy. HR-LC MS and GC-MS were used to target specific extractable PFAS, followed by LC-Orbitrap high resolution mass spectrometry based non-targeted analysis (NTA). Across 42 samples, PIGE analysis revealed that 50% of samples contained < 50 ppm total F, 7% contained 50 - 170 ppm, and 43% > 170 ppm. The highest total F (1770-2470 ppm) was measured in molded "compostable" plant-fibre bowls. Targeted analysis of 9 high-F samples revealed 4 to 14 individual compounds in each sample, with 6:2 fluorotelomer methacrylate and 6:2 fluorotelomer alcohol dominating most samples. 22 compounds belonging to 6 different classes were detected by NTA, 20 of which were measured for the first time in FCMs. We confirmed the widespread use of PFAS and discovered a wide range of previously undetected compounds in plant fibre based fast food FCMs. These compounds could be present as a result of intentional addition to confer grease and water repellency, transformation of other compounds, or as impurities associated with manufacturing, and many more could remain undiscovered due to the specificity required in analytical techniques. This study raises concerns about the use of PFAS in FCMs in terms of human and environmental exposure where the use of PFAS-impregnated "compostable" bowls represents a "regrettable substitution" of single-use plastic FCMs.

04.08.05 Suspect and Non-Target Screening of Reuse Water by Large-Volume Injection Liquid Chromatography and Quadrupole Time-Of-Flight Mass Spectrometry

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As many as 4 billion people are impacted by severe water scarcity at least one month out of the year. As water scarcity increases with a growing global population, water reuse will be important to mitigate water shortages. Therefore, it is necessary to the examine the scope of contaminants in reuse water to ensure that it is fit-for-purpose. Toward that end, eight samples were obtained to characterize the contaminant load in water recycled for reuse applications. The sources included stormwater, rooftop runoff, wastewater, mixed water, and drinking water as a comparison. The water was reused for irrigation, cleaning, toilet flushing, and cooling purposes. Large-volume injection (650 µL) high-performance liquid chromatography and quadrupole time-of-flight mass spectrometry were employed to separate and detect features by suspect and non-target screening. The instrumental method had the advantage that no sample extractions were required prior to analysis. Two chromatographic methods were developed to separate positive- and negative-ionizing compounds and retention time models were developed for both. Retention time models provide an additional measure of confidence for probable and tentative identifications. The two models had predictive R2—which indicates how well the models predicts new observations—of 0.87. After data-reduction, the number of features detected in the samples ranged from 304 to 1513. Feature metrics such as the average response-per-feature provided a simple method to characterize similarities and differences between samples. Additionally, a statistical comparison was performed by principal component analysis. Of the 97 suspect-screening compounds, 20 were positively identified. Benzotriazole/benzothiazole-derivatives and per- and poly-fluoroalkyl substances were the most frequently detected compounds during suspect screening. Other compounds detected included pharmaceuticals, drug metabolites, and sucralose. Features were prioritized for non-target analysis based on in-house library matches, magnitude of response, and frequency of occurrence. Fifty-five unique compounds were positively identified via non-target analysis. The identified compounds included 17 pharmaceuticals, 17 pesticides, 13 industrial compounds, four personal-use compounds, and four biological compounds.

04.08.06 A Data Processing Workflow to Identify Structurally Related Compounds in Petroleum Substances Using Ion Mobility Spectrometry-Mass Spectrometry

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The rapid compositional characterization of petroleum substances is highly desired for informing both its chemical properties and potential hazard; however, currently it is extremely difficult to perform due to the thousands of diverse species present. Ion mobility spectrometry coupled with mass spectrometry (IMS-MS) is a post-ionization separation technique that can be used for rapid multidimensional analyses of complex samples. IMS-MS offers untargeted analysis, including ion-specific conformational data derived as collisional cross-section (CCS) values. Here we combine drift tube IMS CCS evaluations ($^{DT}CCS_{N2}$) and Kendrick Mass Defect (KMD) analyses based on CH_2 and H functional units to enable compositional analyses of petroleum substances. First, polycyclic aromatic compound standards were analyzed by IMS-MS to demonstrate how CCS assists identification of isomeric species in homologous series. Next, we used case studies of a gasoline standard previously characterized for paraffin, isoparaffin, aromatic, naphthene, and olefinic (PIANO) compounds and a crude oil sample to demonstrate the application of the KMD analyses and CCS filtering. Finally, we propose a workflow that enables confident molecular formula assignment to the IMS-MS-derived features in petroleum samples. Collectively, this work demonstrates how rapid untargeted IMS-MS analysis and the proposed data processing workflow can be used to provide confident characterization of hydrocarbon-containing substances.

04.08.07 Chemical Space Visualization for Non-Targeted Analysis via Kendrick Mass Defect Plots and Van Krevelen Diagrams

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The advancements in high-resolution mass spectrometry have led to the development of non-targeted analysis (NTA) methods to assess environmental contaminants of emerging concern, which are not commonly monitored for but are of great importance especially when there is no previous knowledge on the identity or composition of the pollution source. However, the chemical space with respect to NTA is not well defined nor fully understood. In this work, our goal is to understand the chemical space through the use of a Kendrick Mass Defect (KMD) plots and Van Krevelen diagrams (VKD). A KMD plot for NTA data interpretation offers a way to visualize data by adding another dimension to the mass spectra and simplifying the identification of ion(s), helping reduce the massive spectral data usually obtained by restricting compounds within the same homologous series to, for example, a fixed 14 mass unit interval (CH_2) as they tend to share the same KMD. This leads to a simpler plot in which distinctive patterns for homologous organic compounds can be observed. However, an issue with KMD plots is that it can be difficult to identify these homologous series in complex mixtures with many features. An additional graphical analysis method that is often used in conjunction with KMD plots is the VKD (elemental ratio compositions). In our innovative approach to better understand the visualization of the chemical space, KMD and VKD was done by using multiple classes of compounds initially taken from the EPA's DSSTox database. Chemical compounds belonging to the same homologous series tend to have very similar KMD and important patterns can be seen with closely related chemical compounds clustering in linear patterns. However, the same series will have a different appearance in the VKD. Using this method, we are proposing to define anthropogenic regions in the Van Krevelen diagrams to categorize distinctive patterns for compounds with varying chain lengths such as polyethylene glycol (PEG)/polypropylene glycol (PPG), polybrominated diphenyl ethers (PBDEs), organochlorine pesticides, per- and polyfluoroalkyl substances (PFAS), polysiloxane, bisphenol and phthalates, surfactants, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs). These theoretical regions could be used to identify them in environmental samples. The same exercise will also characterize exclusion areas where no known elemental composition exists in the current online databases.

04.08.08 Identifying Persistent Organic Pollutants and Their Metabolites Using Ion Mobility Spectrometry - Mass Spectrometry

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Persistent organic pollutants (POPs) are xenobiotic chemicals of global concern due to their long-range transport, persistence, ability to bioaccumulate, and potential to have negative effects on human health and the environment. Common POPs include a range of diverse chemical classes such as per- and polyfluoroalkyl substances (PFAS), pesticides, polychlorinated biphenyls (PCBs), pharmaceuticals, and byproducts of industrial processes. Identifying POPs in both the environment and human body is therefore essential for assessing potential health risks. Currently, various chromatography approaches coupled with mass spectrometry (MS) including gas, liquid and supercritical fluid chromatography, are the most common analytical methods used to evaluate both parent POPs and their respective metabolites and/or degradants in samples ranging from drinking water to biofluids. Unfortunately, different types of analyses are commonly needed to assess both parent and metabolite/degradant POPs from various classes which presents a number of technical and logistical challenges when rapid analyses are needed, and sample volume are limited. To address these challenges, we characterized compounds from various classes of POPs and their metabolites and/or degradants using ion mobility spectrometry coupled with MS (IMS-MS). Different ionization sources including electrospray ionization (ESI) and atmospheric pressure photoionization (APPI) were employed to determine optimal ionization for each chemical. For assessment of PCB, PAH and pesticides, APPI [+] was necessary for their detection. However, analyses of the PCB and PAH metabolites preferred ESI[-]. For other common POPs such as PCPP and some industrial byproducts found in wastewater, ESI[+] or [-] may be sufficient for analysis of both parent and metabolite/degradant products and was noted to have higher sensitivity than APPI. Additionally, we observe separation of isomeric species including hydroxyl and sulfate PCB, linear and branched PFAS. Collectively, this study advances the field of exposure assessment by characterizing the analytical features of a large number of important environmental pollutants, information that can be used for rapid screening and identification of chemicals in complex samples using rapid IMS-MS.

04.08.09 Unifying Analytical Platforms for PFAS Analysis in a Forensics Context

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PFAS contamination in the environment comes from many sources including municipal landfills, wastewater treatment plants, and aqueous film forming foam (AFFF) applications. Attributing PFAS to specific sources requires an analytical method that works for a wide variety of PFAS with a variety of hydrophobicites and functional groups in aqueous source matrices, without introducing bias. Robust QA/QC is also required to convey confidence in data across the matrices so that statistical analyses identify key components that differentiate sources and not analytical artifacts. Because it is difficult to identify a blank for most of these matrices, it is challenging to define the limit of detection, accuracy, and precision. Thus, a unified method for extracting and quantifying target and suspect PFAS was created for aqueous matrices including source-zone groundwater impacted by AFFF, municipal landfill leachate and wastewater effluent, biosolids leachate, and AFFF concentrates. Small-scale liquid-liquid extraction was employed for matrices where concentration was needed in order to avoid bias introduced by solid phase extraction sorbents. An alternative method was created to determine the method limit of detection and quantification for 50 target PFAS in each of these environmental matrices that had varying background PFAS concentrations. In addition, an alternative method for quantifying suspect PFAS was devised to allow for expansion over time as the number of target and suspect PFAS grows. The NIST Suspect List of Possible Per- and Polyfluoralkyl Substances was also used to identify suspects since each PFAS has a unique identifier. Salt addition and acidification were applied to all aqueous matrices except landfill leachate to improve the recovery of C3-C16 PFAS with a diverse number of head groups. Limits of detection/quantification were determined by substituting overspiked surrogates for native PFAS in non-blank matrices. A single 'master' calibration curve consisting of all target and surrogate PFAS was created to estimate suspect PFAS concentrations. A discussion of uncertainty about estimate concentrations will be included. The extraction and semi-quantification method allows for analysis of a wide variety of PFAS across many complex aqueous environmental matrices and is being applied in a forensics context for source apportionment using continuous variable analysis tools.

04.08.10 Assessing Coastal Plain Groundwater Vulnerability to Extreme Storm and Flood Events Using High Resolution Mass Spectrometry

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Communities in the North Carolina Coastal Plain (NCCP) depend on safe and reliable groundwater for private domestic wells, agriculture, and industry. Although storm intensity and frequency are predicted to increase in coastal areas, the risk of surficial and confined aquifer contamination from extreme storms is not understood. In September 2018, Hurricane Florence caused extensive flooding across the NCCP for several weeks. The North Carolina Department of Environmental Quality (NCDEQ) had just completed sampling of some wells in their monitoring network when Hurricane Florence made landfall. NCDEQ returned to these wells, particularly those flooded by Hurricane Florence, for post-flood sampling. These groundwater samples were analyzed by NCDEQ for regulated semi-volatile organics using standard full scan single quadrupole mass spectrometry (EPA Method 8270). NCDEQ provided NC State the same sample extracts for analysis by high resolution mass spectrometry (HRMS) using a non-targeted and suspect screening workflow with an Agilent 7200 QTOF GCMS. The paired analyses by NCDEQ and NC State continued over a two-year study period and included 150 groundwater samples. Some monitoring well sites experienced flooding during the study period and some did not. The goal of this research was to advance our understanding of coastal aquifer susceptibility to flooding by producing the first comprehensive organic chemical profiles of NCCP aquifers and by determining if aquifers have distinct organic chemical profiles that change after flooding events. Using HRMS, the mean total chemical feature count per groundwater sample was 5,207 (±1,088). Across all groundwater samples, a total of 396 unique chemicals were tentatively identified using the NIST 20 mass spectral database (M^1). Aquifers had distinct chemical profiles, but the range of log K_{ow} values for chemicals detected was similar across all aquifers (approximately 1-8). Chemical profiles of flooded wells, in confined and surficial aquifers, had several regulated organic compounds that were not detected prior to flooding. Comparing pre- and post-flood samples, the number of chemicals of emerging concern detected in post-flood samples increased more frequently for well sites that experienced flooding than for nearby reference well sites that did not flood. Overall, HRMS analyses suggest the intrusion of young water to confined aquifers and the vulnerability of coastal aquifers to contamination from flood events.

04.08.11 Development and Evaluation of NIST Reference Materials Containing Per- and Poly-Fluorinated Alkyl Substances (PFAS)

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Per- and polyfluoroalkyl substances (PFAS) are now widely detected in numerous environmental compartments. For example, PFAS-containing aqueous film-forming foams (AFFFs) used for spill or crash events and during training for fire-fighters has led to extensive soil and groundwater contamination, often impacting drinking water sources and contaminating foodwebs. The need to monitor and assess PFAS contamination in a wide variety of matrices (e.g., water, sediment, tissue) and at a wide range of concentrations (e.g., low-level drinking water; high-concentration groundwater contamination) presents a significant measurement challenge. In response, the National Institute of Standards and Technology (NIST) has established measurements for PFAS in existing Standard Reference Materials (SRMs) (e.g., fish tissue – SRM 1947 Lake Michigan Fish Tissue; 1957 Organic Contaminants in Non-Fortified Human Serum (Freeze-Dried); SRM 2585 Organic Contaminants in House Dust), and is currently developing a new suite of PFAS-specific reference materials (RMs), including contaminated meat tissues (beef steer, dairy cow, and pig), a low-level drinking water material, four AFFF formulations, and a new fish material sourced from Lake Ontario with higher PFAS levels than in SRM 1947. Future efforts will produce RMs for high-level contaminated soil/sediment materials. Beyond the targeted, quantitative measurements typically supported by these RMs, the measurement community also now extensively uses non-targeted analysis to detect and identify novel PFAS for which analytical standards are not available. To support this measurement space, a case study is on-going to explore the potential to include qualitative identifications and semi-quantitative concentrations on the AFFF RM certificates of analysis. The case study will leverage parallel analyses via targeted LC-MS/MS and non-targeted LC-HRMS (quantitation of 41 PFAS analytes; identification and semi-quantitation of additional PFAS), with direct comparisons of instrument performance for quantitative and semi-quantitative analyses. The results will inform analyte inclusion on subsequent certificates with qualitative and semi-quantitative PFAS measurements, are expected to support broader utilization of (S)RMs by the community, and will provide an informative comparison of low- and high-resolution instrument performance for PFAS measurements.

04.08.12 An Inventory of Industrial Effluent and Impacted Water by Non-Targeted Analysis Finds PFAS - Old and New

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Industrial producers and users of per- and polyfluorinated alkyl substances (PFAS) are 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 replacement species. Knowledge of emerging chemical contaminants is of significant environmental and human health impact due to the potential for widespread release, persistence, and bioaccumulation of PFAS species, along with their concomitant toxicological effects, which are frequently understudied. We obtained samples of industrial effluent from a NJ fluoropolymer manufacturer which were emitted to the Delaware River and a local wastewater authority, as well as influent "non-contact" ground water and treated water from the wastewater authority. NTA using high-resolution mass spectrometry was used to examine the chemical identities of PFAS within these samples and compare the chemical identities and abundances of legacy and emerging chemical contaminants. The non-targeted analysis was able to identify legacy contaminants of use in the region [primarily perfluorononanoic acid (PFNA)], a previously identified family of chlorinated perfluoropolyethers (ClPFPECAs), polyfluorinated side products of polyfluorovinylidine (PVDF), as well as several other novel PFAS species exhibiting multiple ether linkages and carboxylic acid head groups. Several of these families have been previously reported as components of fluoropolymer manufacture in other locations in the United States, while some are novel to this measurement inventory. Abundance measurements indicate the measurable presence of effluent derived contaminants in localized groundwater and recirculation through processes that are "non-contact;" localized treatment options designed for PFNA appear to be able to remediate wastewater for emerging contaminants as well.

04.08.13 Non-Targeted Analysis Study Reporting Tool: A New Framework to Improve Reproducibility and Transparency

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Non-targeted analysis (NTA) mass spectrometry methods are increasingly used in diverse fields such as medicine, food science, and environmental health science. Varied research goals have given rise to many complex and distinctive NTA workflows that support specific user needs. Current guidance addresses only limited elements of NTA reporting, and there remains no universal, widely accepted reporting standards for NTA studies. To address this need, the Benchmarking and Publications for Non-Targeted Analysis (BP4NTA) working group developed the NTA Study Reporting Tool (SRT), the first easy-to-use, interdisciplinary framework for comprehensive NTA methods and results reporting. The SRT is organized by study chronology, relying on a structure of sections, categories, and sub-categories, with assigned scores and accompanying rationales based on the quality of reporting in each sub-category. The SRT encompasses all aspects of NTA study design, data acquisition, data processing and analysis methods, data outputs, and quality assurance/quality control (QA/QC) metrics. Using a preliminary version of the SRT, eleven NTA practitioners reviewed eight published manuscripts covering environmental, food, and health-based exposomic applications. During this evaluation, three scoring systems were assessed. Reviewer feedback directed an evolution of the SRT to resolve ambiguity, address coverage gaps, and select a final scoring system. Evaluation results highlighted NTA areas where current reporting practices need significant improvement, most prominently QA/QC metrics. Scores assigned in the two SRT sub-categories assessing QA/QC metrics were both lower and more variable than scores assigned in other SRT sub-categories. Importantly, 72% of scores self-assigned by authors of the evaluated manuscripts fell within the range of triplicate peer-assigned scores, indicating the SRT offers a functional and valid framework to evaluate the reporting quality of key NTA study aspects. The SRT will be available on the BP4NTA website (www.nontargetedanalysis.org/SRT), allowing community access/use and facilitating its continued evolution based on user feedback and future NTA research needs. Widespread implementation is anticipated to improve the efficiency and rigor of NTA study design and review (for manuscripts and proposals), and ultimately, encourage the scientific transparency necessary for utilization of NTA study data by risk assessment and regulatory communities.

04.08.15 Comprehensive Fingerprinting of Polycyclic Aromatic Compounds in Environmental Samples

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Pavement seal coats are black, viscous liquids that are applied to driveways and parking lots in North America to improve the appearance and to increase the longevity of asphalt surfaces. The two most common products are (a) coal tar-based sealant (CTSC) and (b) asphalt-based seal coat (ASC). Studies to date indicate that the concentrations of certain polycyclic aromatic hydrocarbons (PAHs) are approximately 1000 times higher in CTSC than in ASC. Polycyclic aromatic compounds (PACs) are a diverse collection of compounds that not only include PAHs but also alkylated PAHs, sulfur-, nitrogen-, and oxygen-containing PACs. Some PACs exhibit greater mobility and bioavailability in the environment than PAHs due to their increased solubility in water, which in turn increases the risk of exposure to aquatic biota and potentially human exposure through groundwater contamination. The aim of this study is to comprehensively identify and measure PACs in environmental samples and assess what proportion of PACs are from coal tar-based products compared to other sources including asphalt sealants, roofing material tires, and road rubbers diesel particulate. Preliminary profiles have been obtained for 14 different source-based materials using two-dimensional gas chromatography high-resolution mass spectrometry (GCxGC HRToFMS). The 16 native PAHs were observed to be higher than the alkylated PACs for most of the CTSC samples analyzed. On-going efforts are on the way to apply source-apportionment models to assess source contributions of different source-based materials in environmental samples.

04.08.16 Expansion of Nontarget Screening Capability via In Silico Prediction of Indoor Transformation Products

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Impressive strides in nontarget analysis have been achieved in recent years in the identification of previously unrecognized contaminants. Here we further expanded the scope of nontarget analysis to screen indoor transformation products by establishing an *in silico* spectral database, starting with organophosphate compounds (OPCs). First, we used five predominant indoor reaction pathways to predict the indoor transformation products of 713 seed OPCs curated from the Toxic Substances Control Act (TSCA) database. Retention time and MS^2 fragments of each product were further predicted using 13 known OPCs for which reference standards were available. This resulted in an *in silico* mass spectrometry database consisting of 7,710 OPCs with 70,290 corresponding predicted MS^2 fragments. Next, a one-step screening algorithm was applied to identify OPCs in house dusts by matching against the *in silico* database. 44 OPCs were annotated, among which 28 compounds might be formed via indoor transformations where oxidation and hydrolysis play major roles. Finally, we used ^{18}O labeling to confirm that hydrolysis is a major indoor transformation pathway for OPCs and other compounds. This presentation will also describe how these techniques can be expanded to screen transformation products of other classes of indoor contaminants.

04.08.17 Accurate Structural Assignment Tool for PCBs Congeners, Using GC (Front End) and Mass Spectrometric Ortho-Effects

L. Osemwengie, U.S. Environmental Protection Agency / Public Health Chemistry

This study evaluates the use of comprehensive two-dimensional gas chromatography (2D-GC) coupled with a time-of-flight (TOF) mass spectrometer for the separation of 209 PCB congeners, using a sequence of 1D and 2D chromatographic modes. Some of the chlorinated biphenyls that could not be resolved chromatographically are resolved with the use of mass spectrometric "ortho effect," which distinguishes PCB isomers having 2,2'-; and 2,2'6- chlorine substitution from those isomers without these substitutions. Commercially available standards permitted the test of all 209 PCB congeners for the mass spectrometric "ortho effect," or the enhancement of the [M-Cl]+ ion abundance, relative to the molecular ion for biphenyls having 2,2'-; 2,2',6-; and to a smaller degree, 2,2',6,6'-chlorine substitution. Using chromatographic evidence, this study verified that much smaller "ortho effects" were observed for PCBs lacking any, or one ortho chlorine (2-), or two ortho chlorines on the same ring (2,6-). The result of this work potentially provides investigators with a combined new tool for a better front-end (GC column) and mass spectrometric separations of PCB-specific congeners, optimized for the acquisition of more accurate data.

04.08.18 Target and Nontarget Analysis of Per- and Polyfluoralkyl Substances in Wastewater from Electronics Fabrication Facilities

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The goals of this study were to improve our understanding of the types of PFASs that occur in wastewater from electronics fabrication facilities and to assess the relative concentrations of PFAS species. We collected wastewater samples from three electronics fabrication facilities (fabs) in the United States. We analyzed samples by means of high-resolution mass spectrometry and implemented complementary target and nontarget screenings. Twelve of the 25 target PFASs were quantified in at least one wastewater sample. Five perfluorocarboxylates and perfluorobutane sulfonate were quantified in all samples at concentrations ranging from 4 ng L^{-1} to 8,040 ng L^{-1}. GenX and perfluorobutanoic acid were also measured, although exclusively in midstream and downstream samples. The sum of the target PFAS concentrations in the discharge samples from each fab were 623 ng L^{-1}, 394 ng L^{-1}, and 376 ng L^{-1}. A nontarget screening was performed with a data-mining software that extracted series of CF_2, C_2F_4, CF_2O, and C_2F_4O homologs. Nontarget screening revealed the presence of 41 homologous series comprising 131 homologues. We propose structures for 15 homologous series, which included saturated and unsaturated alcohols, ethers, and carboxylic acids, six of which are reported here for the first time. A semi-quantitative approach was used to estimate concentrations of nontarget PFASs and demonstrated that the abundance of some classes of nontarget PFASs, including unsaturated polyether carboxylates, were higher upstream than downstream along the flow path, suggesting possible degradation of these precursor compounds. Other nontarget classes, such as the ether alcohols and carbolxylates, depicted the opposite trend, suggesting possible formation along the flow path. These novel PFASs classes are suspected to be possible transformation products created during the electronics manufacturing process or conveyance. A semi-quantitative approach demonstrated that the sum concentration of all nontarget PFASs was greater than the sum concentration of all target PFASs in eight of the nine wastewater samples. We estimate the sum of target and nontarget PFAS concentrations in the discharge samples from each fab were 1,490 ng L^{-1}, 78,700 ng L^{-1}, and 2,170 ng L^{-1}.

04.08.22 High Resolution Mass Spectrometry (HRMS) Techniques for Screening of PFAS in Environmental Samples

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Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic compounds that are ubiquitous environmental pollutants. These compounds have been widely detected in environmental and biological samples around the globe. It is estimated that there have been 4000 – 6000 PFAS-like compounds manufactured since their introduction with very little toxicological and environmental fate information known. Currently, the most commonly employed analysis for PFAS utilizes a targeted approach utilizing tandem quadrupole mass spectrometers. A targeted approach provides sensitive detection for PFAS, but typically focus on a small portion of potential PFAS of interest. A non-targeted technique can provide a comprehensive characterization of PFAS contamination in a sample. Therefore, the use of analytical methods that can provide data on non-targeted analytes as well as targeted analytes is beneficial and can help to fully assess the environmental distribution of this class of compounds. This presentation will demonstrate various workflows to perform screening of PFAS in water and soil extracts using a high-resolution time-of-flight mass spectrometry (TOF-MS). Use of screening libraries to help identify known PFAS compounds will be demonstrated as well as tools for data interrogation to discover PFAS and PFAS-like compounds that may not be present in a user library, such as common fragment searching, neutral loss, and mass defect filtering. Using this screening workflow, emerging PFAS of interest (PFEESA and PFMBA) were detected in soil and wastewater samples previously characterized using a targeted MS/MS approach that did not contain these compounds in the targeted list.

04.08.23 A Time-Course Analysis of the Sea Water-Soluble Hydrocarbons from a Photooxidized Oil Slick Under Natural Conditions

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Sunlight irradiation can influence the chemical composition of spilled oil and promote dissolution into water thereby altering oil fate. This study aimed to provide a detailed characterization of petroleum photooxidation products collected from underneath a floating oil slick that was exposed to natural sunlight for up to eight days. Oil slicks were generated by adding fresh light crude oil (HOOPS blend, API=35.2) to natural seawater in outdoor mesocosms in the presence and absence of sunlight. The molecular composition of photooxidized oil in the underlying water was characterized over a time course as a function of irradiation status and duration. Analyses included gas chromatography-mass spectrometry (GC-MS), biomimetic extraction-solid phase microextraction (BE-SPME) with GC-flame ionization detection (GC-FID), and ion mobility spectrometry-mass spectrometry (IMS-MS). Exposure to sunlight irradiation resulted in a time-dependent increase in soluble organic acid species in the sea water. As compared to GC, the IMS-MS analysis enabled detailed characterization of parent and oxygen-containing hydrocarbons in the underlying water and showed that mono-oxygenated hydrocarbons increased the most over time and were sunlight exposure-dependent. BE-SPME is known to be correlated to aquatic toxicity and analysis of water samples using this technique indicated a transient, two-fold increase in dissolved photoproducts following irradiation. We conclude that natural sunlight can produce measurable photooxidized hydrocarbons from a floating oil slick under natural conditions and promote their dissolution in seawater.

04.08.25 Creating a Database Infrastructure for the Management and Analysis of Mass Spectrometry Data for Per- and Polyfluoroalkyl Substances

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With thousands of known per- and polyfluoroalkyl substances (PFAS), but a limited number of analytical standards, the detection and identification of most PFAS is still limited to instrumental and computational approaches without the use of validation through chemical standards. A primary step in achieving probable identification of unknown compounds via liquid chromatography with high resolution mass spectrometry (LC-HRMS) is through using mass spectral libraries. While numerous studies have identified novel PFAS using LC-HRMS, often the datasets have not been made publicly available and there is a limited quantity of empirical mass spectra associated with novel PFAS. To address this data gap, researchers at the National Institute of Standards & Technology (NIST) are developing an open-source approach to collecting, managing, and analyzing mass spectral data from contributing laboratories. The status and future objectives of the project will be presented. Using up-to-date databases of PFAS, a list of over 4000 possible PFAS structures has been systematically created for referencing chemical identities and is now publicly available. The underlying database schema has been designed using an open-source format to make all aspects of the data accessible to researchers. Currently, over 400 individual mass spectra, representing over 100 unique PFAS, have been acquired from various sources for the database. As instrumental method information can be essential to understanding the quality of mass spectral matches, researchers are developing tools for parsing and collecting instrumental method metadata. In addition, a novel approach to understanding the variability of measured mass spectra has been developed and will be included in the database for a more comprehensive determination of compound match scores. Finally, the protocol for contributing mass spectral data will be presented to enable further expansion of the database.

04.09 Non-targeted Analysis: Prioritization of Organic Contaminants for Monitoring and Toxicological Studies

04.09.01 Finding Failure: Identifying the Practical Limits of Multivariate Tools in Targeted and Non-Targeted Forensics Analyses

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The fundamental hypothesis underlying chemical forensics analyses is that the chemical composition of samples from a particular source are more similar to samples collected from the same source compared to those collected from a different source. This hypothesis implies that a diagnostic chemical fingerprint could be developed from a subset of chemical features that are produced from each source. Chemical fingerprinting workflows have been called for in the literature, and multivariate computational techniques (e.g., ordination, classification) have been heralded as critical tools in such forensics analyses. While the existing body of literature appears to support this hypothesis, it is important to note that many studies using multivariate tools to analyze chemical datasets do not state the assumptions or discuss the appropriateness or limitations of their chosen multivariate tool(s). Therefore, it is difficult to assess the validity of results published from such studies. Using ten environmental datasets and eight multivariate tools, our goal is to identify the practical limitations associated with multivariate tools when analyzing complex environmental data sets. Environmental datasets will consist of a range of target (e.g., PFAS concentrations, cations/anions) and non-target chemical data with the chemical composition ranging from tens to tens of thousands of chemical features. All multivariate tools and datasets were chosen to capture a broad spectrum of computational techniques and data structures. Identifying the limits of these tools will help scientists apply these tools appropriately and avoid misuse of complex multivariate tools.

04.09.02 Quantifying Untargeted Metabolomic Changes in Watersheds and the Contribution of Putative Environmental Drivers

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Dissolved organic matter (DOM) plays a significant role in watershed ecosystem metabolism and nutrients cycling, with further impacts on water quality and food security. However, the assemblages of tens of thousands of unknown and continuously changing organic molecules in an ecosystem, sometimes referred to as a metabolome, make it challenging to characterize the composition and determine the drivers of variation. The dynamic nature of untargeted metabolome records the information of upstream land-use sources and in-stream watershed processes. By screening a single water sample from the outflow of a watershed, it is theoretically possible to simultaneously quantify all ecosystem processes occurring upstream in a watershed simply by testing for the presence of the diagnostic metabolomic signatures (fingerprints). Therefore, it is critical to identify metabolomic signatures that are indicative of their sources and/or ecosystem processes. Advances in high resolution mass spectrometry (HRMS) and machine learning tools make it possible to decode watershed metabolomes. Our aim is to build upon these strategies to identify the different metabolomic signatures that are indicative of different land-use sources and watershed processes. In this study, we quantified the temporal and spatial variability of environmental metabolomes from 4 watersheds in northwestern Oregon. The preliminary results indicate strong seasonal variability in metabolomic composition. The findings suggest that seasonal ecosystem processes are the leading driver of the metabolomic changes, rather than spatial variation or land-use sources in these four watersheds. Metabolomic signatures of different land-use sources are also identified in each season. Future studies will develop fingerprinting workflow from watershed metabolomes with more diverse land-use sources and ecosystem processes.

04.09.03 Opening Pandora's Box: Machine Learning Applications for Chemical Forensics and Non-Target Chemical Analyses

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Chemical fingerprinting is a forensics analysis that seeks to identify a suite of chemical features that are diagnostic of their source. While such workflows have been called for in the literature and have been described as paradigm shifting, selecting a subset of diagnostic chemical features (e.g., 10-100) out of the thousands of candidate features remains an obstacle. Herein, we report on a simple fingerprinting workflow that quantifies the importance of every chemical feature based on its ability to predict presence of a source. With this workflow, we are able to probabilistically predict the presence of two or more sources within mixed water samples (e.g., surface waters) and to evaluate the relative quality of each fingerprint. Briefly, water/solid samples were collected from municipal wastewater, road runoff, livestock manure, and runoff from agricultural fields. Following sample processing, 8,381 non-target chemical features were retained for classification analysis across all samples. Support vector classification (sklearn.svm.SVC) was programmed in Python to identify the 10, 25, and 50 chemical features that were most predictive of each source. Regardless if 10, 25, or 50 chemical features were used, the fingerprinting workflow was able to distinguish pollution source with near-perfect cross validation accuracy. In addition to source specific samples, surface water samples were collected from local creeks and screened for the presence of each source fingerprint. The wastewater chemical signature was the most abundant signature detected in surface waters, and it was most prevalent downstream of wastewater treatment plants. Other chemical signatures were detected unexpectedly during the dry summer season, but were absent in the winter wet season. This was contrary to our expectation given the hydrology of the system. Although baseline probabilities demarcating presence and absence were generated, each source had a different critical threshold probability, suggesting that some fingerprints were or higher quality. Therefore, limits of detection for chemical fingerprint should be uniquely established for each source. Ultimately, our goal is to use this workflow to identify the chemical sources that have a disproportionally high abundance in surface bodies of water.

04.09.05 Non-Targeted Screening and Distribution of Novel Halogenated Compounds in Great Lakes Top Predator Fish

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Monitoring programs traditionally are tasked with monitoring known legacy contaminants. However, these finite lists of contaminants are inadequate considering thousands of chemicals in commerce and breakdown products of the legacy contaminants. Non-targeted analyses have become more promising with recent advances in analytical instrumentation and data analysis tools. As part of the Great Lakes Fish Monitoring and Surveillance Program (GLFMSP), a non-targeted screening methodology was developed and applied to Great Lakes fish using a 2-dimensional gas chromatography coupled to a high-resolution time of flight mass spectrometer (GCxGC-HR-ToF MS). More than 60 novel halogenated species were identified in fish from all of the Great Lakes, with halomethoxyphenols (MeOPs) representing the most abundant chemical class. With analyzing multiple years of samples, spatial and temporal trends of the unknown features were assessed prior to identifying the features with the goal of prioritizing and identifying unknown persistent halogens in the Great Lakes. Coupled with targeted chemical monitoring in the Great Lakes region chemical profiling provides a means to protect the region from known and unknown emerging contaminants of concern.

04.09.06 Examination of Trends and Variations in Contaminants in Surface Water, Sediment, and Mussel Tissues via Non-Targeted Mass Spectrometry: An Everglades Case Study

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The Contaminant Assessment and Risk Evaluation (CARE) project evaluated traditional contaminants in the Florida Everglades such as metals, nutrients, and organic contaminants. The CARE project was focused directly in the Everglades, and inhabited areas such as Everglades City and Chokoloskee were excluded from the project, making these prime areas for determining contaminant flux. The Barron River in Everglades City receives water from the wetlands to the North, which are fed by the agricultural areas near Immokalee. Previously surface water, mussel tissue, and sediment samples were obtained seasonally in 2018-2019 from the Barron River in Everglades City. Reports of symptoms of potential contamination has renewed interest in the area, spurring the development of a non-targeted screening method for surface water, tissue, and sediment matrices as well as analysis via traditional targeted methods. Tissue extractions for nontarget analysis were achieved through extraction via methanol in a Dionex ASE 200 with a cell using a modified version of a method previously studied for nontargeted extraction of fish tissue by Du et al (2017). Due to the higher fat content of the mussel tissues extra cleanup steps were required. Extracts were chilled at -20^o C for four hours to separate lipids before concentration under N_2 gas at 40^o C and filtered through a 0.45 micron Teflon filter that was prewashed with methanol. Additional dilution of the concentrated extract with LCMS grade water was required to maintain signal of quality control compounds previously used by Ng et al (2020). Diluted extracts were analyzed via liquid chromatography-high resolution mass spectrometry using a Thermo Q Exactive Orbitrap. Samples were analyzed in positive and negative ionization modes in full scan from m/z 100-800 at a resolution of 140,000 and data dependent MS2 for confirmation with an NCE of 30. Data processing was performed using Thermo Compound Discoverer version 3.1. Preliminary bulk data analysis via VanKrevelen and Kendrick's Mass Defect plots show that the tissue extracts are largely similar, although analysis of the southern sites detected more features (n=2081) than the northern sites (n=952). Additional examination of individual features showed a large number of natural features that would be expected in a tissue sample such as l-Isoleucine and adenine present across all sites, and more anthropogenic features such as dibutyl phthalate and azelaic acid in the southern sites.

04.09.08 Identifying the Right Needle in the Haystack: Chemometric Approaches to Determine Statistically Relevant Contaminants in Environmental HRMS Studies

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With over 15,000 new chemicals introduced into commerce each day, and only a 100+ chemicals currently regulated in water in the US, there is a dire need to monitor unknown and emerging contaminants but also prioritize identification as it is not practically feasible to monitor every single one. While toxicological studies are still the gold standard for chemical regulation - these studies can take years and generally involve apriori information of their presence in the environment to prioritize their study. The increasing use of hi-resolution mass spectrometry (HRMS) has provided the environmental community with the ability to identify several hundred untargeted compounds in the environment. Non-targeted methods using HRMS can identify many compounds but several compounds identified may not be important when studying changes in an environmental system such as a water body. Determination of what components are significant and identifying them is critical, but difficult given the variability of environmental samples across time and season. The use of chemometric techniques after collection of HRMS data could allow for the possibility of identifying specific chemicals that are persistent, frequently detected and of most relevance in a particular environmental system while saving significant time by not having to identify all the chemicals detected. This work examined LC/HRMS data of a river influenced by agriculture, and domestic wastewater over 8 months using multivariate statistics to determine compounds that are consistently being discharged into the environment and should be identified and monitored for evaluation and potential prioritization of toxicological studies. Replicate samples of wastewater effluent, downstream, tributaries, field blanks and lab blanks were analyzed. Using chemometrics and statistical tests, the number of relavent features in the samples collected downstream of a wastewater plant compared to the effluent were reduced from several thousand around a hundred without the need for specific identification. Further, Non-targeted analysis using database of >10,000 compounds gave positive identification of some of these compounds that are constantly present in the water after wastewater discharge and require monitoring plus further toxicological evaluation for potential health effects. This analysis also demonstrated that although compounds of interest can be determined with screening databases, there are several compounds that cannot be identified. Hence, the use of structure prediction software to identify some of these true 'unknowns' in the water is needed. With the locations sampled across several months, statistical techniques are used to distinguish unknowns not in a database that appear consistently and thus should be identified to determine their importance for prioritized monitoring. This study provides a template for the identification of 'emerging' contaminants of relevance and for priority identification in an area using statistical processes.

04.09.09 Using Estrogen-Responsive Reporter Gene Assays to Identify Nontarget Mammary Gland Carcinogens in Drinking Water

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Four regions in California have been identified by the California Breast Cancer Research Program and Public Health Institute as regions of disproportionally high rates of breast cancer compared to the rest of the state. This study aims to identify chemicals in household drinking water correlated to measured estrogenicity and/or to these regions of breast cancer "hot spots". Tap water was collected from 120 homes in California and analyzed using High Resolution LC- and GC-QTOF-MS and Chemically-Activated Luciferase Gene Expression estrogen bioassays. In addition, water source types were evaluated amongst the 8 regions investigated (1) large surface water (Tuolumne and Mokelumne rivers), (2) small surface water (Klamath and Trinity/Weaver Creek. (3) ground water (San Joaquin Basin), and (4) mixed ground and surface water (Colorado River and ground water in Southern California). A combination of cell-based estrogen-responsive bioassays are combined with this metadata to prioritize estrogen active features from a nontargeted dataset.

04.09.11 Prioritization and Identification of Bioactive Wastewater Contaminants by Coupling Effects-Based Monitoring and High-Resolution Mass Spectrometry

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Effects-based monitoring uses biological pathway-based tools to identify biological activity (e.g., estrogen receptor (ER) activation), providing a complement to chemical analysis in environmental monitoring. While effects-based approaches are a valuable tool for environmental monitoring, these approaches alone cannot explicitly identify the contaminants inducing biological activity. For example, as part of a case study that monitored bioactive contaminants in surface waters along the Colorado River near Moab, UT, before and after upgrades to wastewater treatment infrastructure, water samples were screened for in vitro biological activities and a targeted list of pharmaceuticals, personal care products, pesticides, and steroid hormones. The most prominent bioactivities observed in the river system were ER, glucocorticoid receptor (GR), and peroxisome proliferator activated receptor gamma (PPARg)-related activity, with PPARg activation occurring less frequently and at a lower relative concentration. Targeted chemical measurements could adequately explain ER-related bioactivity, but not GR or PPARg. To further investigate and potentially identify unknown contaminants contributing to GR- and PPARg-mediated biological activity, high-resolution mass spectrometry (HRMS)-based methods were applied to the same water samples. HRMS analysis of extracts previously shown to induce biological activity was performed using a Thermo ID-X LC-MS system with electrospray ionization. Wastewater treatment infrastructure upgrades significantly reduced bioactivity in receiving waters. Likewise, unknown compounds detected through HRMS methods decreased following infrastructure upgrades. These findings highlight the potential application of bioactivity-focused methods along with HRMS for unknown environmental contaminant identification. *The contents of this abstract neither constitute nor necessarily reflect USEPA policy.*

04.09.13 Occurrence and Bioaccumulation of Legacy and Novel PFAS in the Delaware River Estuary Detected Using Targeted, Suspect, and Non-Targeted Analysis

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With the phase-out of some legacy per- and polyfluoroalkyl substances (PFAS), new PFAS have been introduced in consumer and industrial applications. Little is known about the occurrence, biological accumulation, or potential impacts of novel PFAS. Here, we used high resolution mass spectrometry to evaluate legacy and novel PFAS in surface water, passive samplers, fish muscle, and fish liver from the Delaware River. This region is home to historical and current PFAS producers and users. Samples were collected within the mainstem River and tributaries at sites located between Bristol, Pennsylvania downstream to Elsinboro, New Jersey (NJ), spanning over 50 river miles. Targeted, suspect, and non-targeted analyses were conducted, and features identified using suspect lists and non-targeted analyses were semi-quantified using surrogate normalization or presented as raw abundances. We identified a series of perfluoro-polyether carboxylates (X-PFPECAs) in surface water, passive samplers, and fish tissue from across the sampled area, with the highest abundances adjacent to and downstream from a suspected point source in southwest NJ. Some of the chlorinated and hydrogenated species within this series were previously identified in soil, groundwater, and local surface water; here, we establish for the first time the presence of these and additional chlorinated, hydrogenated, and fluorinated congeners in the wider aquatic ecosystem. Some novel X-PFPECAs were orders of magnitude more abundant than legacy PFAS in each sampled matrix. In white perch liver, the novel Cl-PFPECAs were an order of magnitude more bioaccumulative compared to perfluorooctanesulfonic acid (PFOS) based on semi-quantitative concentrations from surface water and fish liver tissue sampled from the same location. We also describe several previously unidentified PFAS; spectral evidence suggests one novel group incorporates multiple ether linkages and at least two hydroxyl groups. Legacy PFAS including perfluorononanoic acid (PFNA), PFOS, and perfluorooctanoic acid (PFOA) were also identified in surface water and fish tissue, with some fish muscle concentrations exceeding human consumption advisories issued by the state of NJ. This work highlights the continuing evolution of PFAS detection in the environment, and underscores the importance of non-targeted methods to screen for PFAS beyond limited targeted lists.

04.09.14 Non-Targeted Analysis of Pfas-Free Aqueous Film Forming Foams

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Aqueous film forming foams (AFFFs) used for fire suppression typically contain per- and poly-fluorinated alkyl substances (PFAS). These formulations include long-chain compounds such as PFOS and PFOA, although newer formulations have shifted to shorter-chain replacement PFAS (e.g., PFHxS). Recent studies have demonstrated that PFAS not only persists in the environment, but can bioaccumulate, biomagnify, and exhibit sublethal toxicity to fish and aquatic invertebrates. This led to mandated replacement of PFAS-containing AFFFs by October 2023. In response, the Strategic Environmental Research and Development Program (SERDP) requested comprehensive acute and chronic toxicity information on six alternative PFAS-free AFFFs, relative to a current-use short-chain PFAS-containing AFFF. Chemical composition of these AFFFs is proprietary; however, there is a need for analytical methods that allow translation between nominal and actual concentrations of these complex mixtures during exposure, and that support assessment of any AFFF degradation/transformation. Accordingly, liquid chromatography (LC) coupled to non-targeted high resolution mass spectrometry (HRMS) was applied to analyze the AFFFs under investigation, assess stability of foam stock solutions, and isolate reliable "marker compounds" whose occurrence and relative abundance correspond to the nominal concentration of the AFFF mixture. Marker compounds were selected via analysis of gravimetrically prepared dilutions of AFFFs (3 % stock solutions prepared in deionized water; subsequent dilutions in methanol), with data reduction criteria developed to prioritize five to ten features per foam that exhibited linear relative abundance with dilution and were stable over time. Although discrete chemical identities were not assigned, MS/MS analysis of AFFFs yielded Q1→Q3 ion transitions for selected marker compounds that were used to build a targeted LC-MS/MS method (on a triple-quadrupole mass spectrometer) to support accurate foam concentration assessment during toxicity tests. Initial method evaluation indicated linear responses between 0.1 - 30 ppm AFFF (in methanol) for three to five marker compounds per formulation, with actual exposure concentrations within 100-120 % of nominal. Method development details, data reduction parameters, and comparisons of nominal and measured concentrations from the toxicity exposures will be reported and discussed.

04.09.15 Target and Non-Target Screening of Priority Organic Chemicals in Recycled Plastics in Canada

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Increasing global plastic consumption and waste generated has encouraged sustainability initiatives and programs, including demand for recycled plastic. However, there are concerns that recycling of consumer plastics could introduce hazardous chemicals into repurposed plastic products. Our objective was to use target and non-target analysis to characterize contaminants in various recycled products including both flakes (cleaned and chipped original recycled materials) and pellets (produced by heating and extrusion of flakes). We obtained 26 samples, from five Canadian recycling companies, including low- and high-density polyethylene (LDPE and HDPE, respectively) pellets and flakes, polypropylene (PP) flakes, high impact polystyrene (HIPS) pellets, and polyethylene terephthalate (PET) pellets. Target analysis for halogenated flame retardants (Br/Cl-FRs) using GC-negative ion MS showed low ng/g concentrations of polybromodiphenyl ethers (PBDEs). However, PBDE substitutes were present at higher levels (100- 2100 ng/g) with 2-ethyl-1-hexyl 2,3,4,5-tetrabromobenzoate (EHTBB) and hexabromocyclododecane (HBCD) being most prominent especially in colored HDPE and PET pellets. Analysis of perfluoroalkyl acids by LC-MS/MS showed very low levels (< 0.2 ng/g). Non-target analysis was conducted using comprehensive two-dimensional GC coupled to time-of-flight mass spectrometry (GC×GC/TOF-MS) and LC Orbitrap high resolution mass spectrometry (LC-HRMS). Non-target screening across the various products led to the identification of over 100 chemicals by GC×GC/TOF-MS (>80% NIST mass library match), including polycyclic aromatic compounds, benzoic acid esters and benzoates. Many known commercial and industrial antioxidants, stabilizers or plasticizers, such as bisphenol A, octabenzone and several phthalates were detected by LC-HRMS (accurate mass/fragmentation patterns and mzCloud spectral database matching). Judging by the range of concentrations the chemicals appear to be a residual trace contaminants and impurities; most targeted analytes were 103 to 106 fold lower than guideline limits (eg 0.1% or 1 mg/g) for Substances of Very High Concern in products in Europe. An exception was "other Br-FRs", which had concentrations ~ 70 to 100x lower than the European guideline for flame retardants, Given the wide range of chemicals identified, albeit at low levels. we recommend further evaluation of a wider range of recycled plastics and consideration of additive effects.

04.09.16 Non-Targeted Analysis of Phthalate Metabolite in Human Urine by Diagnostic Fragmenention Pathways and Predicted Retention Times

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Phthalates are widely used as plasticizers in polyvinyl chloride (PVC) plastics and solvents in many consumer products. Widespread use of phthalates makes them ubiquitously present in the environment and one of the synthetic organic chemical classes to which humans are most frequently exposed [1,2]. Liquid chromatography - mass spectrometry (LC-MS) systems are commonly used to provide quantitative information for targeted surveillance of well-known phthalate metabolites in urine and exposure assessment. However, well-known targeted phthalate metabolites with commercially available standards represent only a portion of phthalate metabolites in real samples. New phthalate compounds are constantly being generated by industry, and the transformation and metabolism of both new and old phthalates has not been completely characterized. Therefore, there remain many phthalate metabolites which have yet to be monitored for assessment of human exposure. Due to its discovery based nature, non-targeted analysis using high-resolution mass spectrometry (HRMS) is increasingly used to screen and identify previously unknown metabolites as exposure biomarkers [3]. In this study, a non-targeted analysis method has been developed for identification of new phthalate metabolites in human urine. In this method, the three structurally specific ions, namely the deprotonated benzoate ion [C6H5COO]- at m/z 121.0290, the deprotonated o-phthalic anhydride ion [C8H3O3]- at m/z 147.0082, the [C8H5O4]- ion at m/z 165.0188 representing, as a filter were used to quickly prioritize candidate precursor ions associated with phthalate metabolites from the data collected in data independent acquisition (DIA) mode (all ion fragmentation). The four fragmentation pathways were applied to structure interpretation and distinguishing the types of phthalate metabolites identified, and the predicted retention time (RT) of the proposed structure was used to support the interpretation via comparing it with the observed RT. Eight unknown precursor ions were identified in the pooled urine sample, but only one followed the fragmentation pathway. It was interpreted as unreported new phthalate metabolite by the MS/MS spectrum and its predicted retention time supported the interpretation. The developed method can identify new phthalate metabolites in human urine with a detection limit of less than 50 ppb.

04.09.17 Chemical Characterization by Non-Targeted Analysis of Airborne Particulate Matter From South Florida Coastal Area Influenced by African Dust Events

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Airborne particulate matter (APM) are formed by a complex mixture of dust, pollen, soot, smoke, and liquid droplets containing organic, inorganic and biological components as well. Saharan dust storms are responsible for the transport of a large amount of mineral dust across the Atlantic Ocean to the Caribbean, Central, and North America during the summer months. African dust particles can be a great carrier of air pollutants by adsorption (aerosolization), leading to negative impacts in air quality. APM below 10 µm are known to enter the respiratory track and have been associated with severe health effects such as asthma, pneumonia, allergic rhinitis, cardiovascular diseases, among others. When the African dust reaches Florida, US, more than 50 % of the dust are smaller than 2.1 µm. Although previous studies have addressed metals and pesticides in APM, there is a need to better understand the chemical composition of APM regarding organic contaminants of concern. Thus, this study focuses on the fingerprinting of organic chemicals by non-target screening of dust samples collected during high African dust events in the wet season, as well as in the dry season in South Florida area. Here, an air sampler located at the top of the Marine Science building at FIU, Biscayne Bay Campus, was used with Versapor filters, collecting APM from June 2020 to March 2021. Filter particulate load was measured gravimetrically, and dust samples were extracted with methanol and analyzed by liquid chromatography-high-resolution mass spectrometry using a Q-Exactive Orbitrap. Raw data was processed using the Compound Discover v.3.1 software, including the process of peak picking, blank subtraction, merging and grouping of features, molecular formula generation, isotopic pattern comparison, evaluation of adducts, the assignment and comparison of fragmentation pattern, and the searching of databases. A list of features containing molecular formula, retention time and name assignment by database match will be generated and the tentatively identified compounds will be grouped into different classifications, such as phthalates, pesticides, pharmaceuticals, perfluoroalkyl substances, etc. Method performance will be assessed in terms of accuracy, precision and selectivity by prepared in-house quality control and labeled standard mixtures. This study will provide detailed information on unique chemicals transported by African dust and their potential impact on the environment and humans.

04.09.19 A Framework for Utilizing High Resolution Mass Spectrometry and Non-Targeted Analysis (NTA) in Rapid Response and Emergency Situations

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Chemical releases of unknown compositions occur daily, with over 9,000 calls reporting unknown discharges to the environment logged by the National Response Center (NRC) in 2019 alone. By the very virtue of their unknown identity, these releases pose potential risk to human and ecological receptors in the affected areas and cause unique challenges for rapid responders and risk managers called on to characterize, contain, and remediate contamination associated with the event. With a variety of mobile assets bringing analytical capabilities to the site of the event, a broad network of chemists and brick-and-mortar analytical laboratories, and dedicated on-scene coordinators to monitor and direct response efforts, the U.S. Environmental Protection Agency (U.S. EPA) plays a key role in responding to chemical releases in rapid response scenarios. Current approaches used to characterize unknown chemical releases include a suite of low-resolution targeted and screening methods. Several cheminformatic tools developed (or currently in development) at the U.S. EPA, enabling semi-automated NTA workflows necessary for quick data turnaround times, have made NTA more applicable to emergency risk management. As such, high resolution mass spectrometry (HRMS)-based non-targeted analysis (NTA) approaches could improve confidence in chemical identifications made to support on-scene decision making and, in some cases, facilitate quicker response times when a release of an unknown substance occurs. While rarely used in current rapid response protocols, the application of NTA has clear precedent in research involving drinking and surface water monitoring, assessments of illicit drug intake in clinical settings, and both long and short-term evaluation of contaminant mobilization following public health and natural disasters. In light of these examples and recent cheminformatic advancements, we propose a framework for utilizing HRMS and NTA in rapid response and emergency situations. In future efforts, a series of mock scenarios will determine the practicality of our proposed framework. Ultimately, this research will better educate NTA practitioners about the data requirements and timelines governing an effective response, while also disseminating key NTA tools to chemists that support emergency responses.

04.09.20 Fluorescent Spectrometry for Rapid Non-Targeted Analysis of Polycyclic Aromatic Hydrocarbons and Their Metabolites in Bioremediation Studies

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Polycyclic aromatic hydrocarbons (PAHs) are a class of environmental contaminants that are ubiquitous in the environment and pose health risks to both humans and ecosystems. PAHs can undergo biotic degradation, and therefore exist in the environment as a complex mixture of parent PAHs and PAH metabolites. PAHs and PAH metabolites require extensive analytical techniques to extract, separate, and identify, making them difficult to monitor in real time. Furthermore, these analytical efforts are often hindered by the lack of, or costs associated with, standards available for the multitude of compounds present in a complex mixture. Fluorescent spectrometry can be used as a non-targeted analysis to monitor parent PAHs and their metabolites in aqueous matrices. While scan parameters can be optimized for the detection of specific compounds known to be present, scan parameters can also be generalized to detect unknown compounds in a sample. Two-dimensional and three-dimensional scans produce emission spectra and excitation-emission matrices (EEMs) respectively that can be used to derive pseudo-first order transformation rates, monitor reaction progress, and reveal differences in the composition of metabolite mixtures. In this study, two pure cultures, *Mycobacterium* Strain ELW1 and *Rhodococcus rhodochrous* Strain 21198 were used in transformation studies of parent PAHs. Fluorescent scans were used to monitor reaction progress, time sampling events for targeted analyses, and demonstrate different transformation abilities between the two microorganisms. Shifts in fluorescent spectra indicate that while both pure cultures are able to transform the parent PAHs, different metabolite mixtures are formed. Fluorescent spectrometry offers the ability to monitor the transformation of parent PAHs by microorganisms in real time with minimal sample preparation and data analysis required. This method can be used to screen microorganisms for PAH transformation capabilities and inform sampling schedules to prioritize samples for targeted analyses.

04.10 Passive Sampling: Recent Advances and Application

04.10.01 Field Validation and Application of a Novel Passive Sampler to Determine Sources and Sinks of PFAS in an Urbanized Estuary

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Several per- and polyfluoroalkyl substances (PFAS) are of growing concern worldwide, due to their ubiquitous presence, bioaccumulation and adverse effects. Surface waters in the United States have displayed elevated concentrations of PFAS, but so far discrete water sampling has been the commonly applied sampling approach. Here we field-tested a novel integrative passive sampler, a microporous polyethylene (PE) tube, and derived sampling rates (R_s) for 9 PFAS in surface waters. Three sampling campaigns were conducted, deploying PE tube passive samplers in two wastewater treatment plant (WWTP) effluents and across Narragansett Bay for one month each in 2017/2018. Passive samplers exhibited linear uptake of PFAS in the WWTP effluents, with in-situ Rs ranging from 18 mL day^{-1} (PFPeA) to 43 mL day^{-1} (PFHxS). Applying the passive samplers in a different WWTP yielded an agreement between derived and measured effluent concentrations within 31%, while larger discrepancies were observed across the estuarine deployments. Extrapolating PFAS emissions across all WWTP discharging into the estuary implied a daily input of ~ 77±6 g S_9PFAS daily, with sedimentation only removing ~ 2 g daily, though more important for the longer-chain compounds (≤ 10% removal). These results highlight the potential use of passive samplers as monitoring tools of PFAS in dynamic aquatic environments.

04.10.02 Optimization of Passive Sampling Applications Using Mass Transfer Modeling

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Polymeric passive sampling provides a time-integrated measurement of freely dissolved concentrations of hydrophobic organic contaminants (HOCs), reflecting the mean exposure concentration to the ecosystem receptors. However, in the real environment, water concentrations of these HOCs vary temporally with events like stormflow, industrial runoff, and tides. The effect of such events on the time-weighted-average water concentration is important for ecological exposure assessment. In this study we simulated a range of conditions for ambient concentration of the freely dissolved polychlorinated biphenyl (PCB) molecules, sampler characteristics, and molecular properties to answer some relevant questions pertaining to how well passive sampler concentrations represent the time-averaged concentration over an entire deployment period: Is there a time-period beyond which a perturbation in concentration is not captured in the passive sampling time-integration? If yes, how does the different characteristic time scale of integration vary for different compounds based on hydrophobicity or the physical characteristics of the sampler. Can we capture the true water concentration of PCBs within a day after a perturbation with a suitable PE thickness under very turbulent conditions (so that the uptake is fully PE-controlled)? Are these predictions consistent for the various models simulating the mass transfer kinetics? To answer these questions, we use both first order and one-dimensional Fick's diffusion model to simulate the uptake and loss kinetics of polychlorinated biphenyl (PCB) molecules in a polyethylene (PE) sheet from water. These include simulations of 1) varying day of introduction of a 10-day long perturbation in ambient PCB concentration, 2) the effect of the ambient perturbation on the uptake of different PCB homologs, 3) different sampler thickness. The uptake and loss kinetics of target analytes and the corresponding PRCs from various thicknesses of PE within a day into a highly turbulent infinite water bath was modeled by the Diffusion model and solved both numerically and analytically. Results from these simulations were interpreted to enable better understanding of the nature of time-integration in passive sampling and allow tailoring of sampler design and deployment to target a desired period of time-integration.

04.10.03 Use of Active In Situ Samplers for Organic Pollutant Assessment: Field Applications and Laboratory Comparisons

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Discrete grab samples from the water column for contaminant analysis can suffer from several methodological drawbacks. For example, constituent concentrations in marine environments can change quickly with tides, currents, and runoff events, meaning that a discrete water sample only represents a "snapshot" that may not be temporally representative of the system. Additionally for some analytes, large volumes of water may be required in order to detect the chemicals; this may not be logistically feasible, especially at remote field locations. *In situ* time integrative sampling devices have the potential to address these issues. *In situ* active samplers (CLAM devices) were used to assess organic chemical contamination of coral reef ecosystems in Fagatele Bay, part of the National Marine Sanctuary of American Samoa. This system has significant concerns about the potential inputs of contaminants to the Bay from the Futiga Dump landfill. Leachate from the landfill could reach the Bay through groundwater or surface runoff. This study produced a baseline chemical characterization of Fagatele Bay for over 100 chemical contaminants in the water column using *in situ* active devices at eight sites within the Bay. Chemistry results show that while there was detectable contamination within the Bay, levels were low compared to published toxicity values. This suggests anthropogenic chemicals are reaching the coral reef ecosystem at sublethal levels, which could be causing subtle effects, and further study should target source identification and reduction. Future monitoring of the Bay is warranted to detect potential change in stressors and reef resources. This study also showed that CLAM devices are useful as an alternative to grab samples for measuring time integrated water quality in coral reef ecosystems. These active samplers were also compared with grab samples and two types of recognized passive samplers (POCIS and silicone bands) in a mesocosm laboratory study for selected analytes (triclosan, pyrene and biphenthrin) in known concentrations. The mesocosm assessment showed that both passive and active samplers may have utility for marine contaminant applications, but the most appropriate method will be determined by the research question, field logistics and budget considerations.

04.10.04 Evaluating the Sorption Kinetics of HOCs in Activated Carbon by Batch Experiments Using Polydimethylsiloxane Passive Samplers

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Activated carbon (AC) has been applied widely in environmental engineering as a strong sorbent to organic contaminants. Commercial AC is normally available as powdered activated carbon (PAC) (typically passing 80 mesh, 0.177 mm) and granular activated carbon (GAC), 0.5-1 mm or larger in size. Depending on the particle size and the intraparticle diffusivity of target compounds, the characteristic time for adsorption in AC may vary from minutes to years and lead to significant differences in adsorption performance in applications such as in-situ sediment remediation, amended sediment capping or wastewater treatment. The sorption kinetics in a given AC is commonly evaluated by batch experiments that measure the change of water concentration over time after placing clean AC in a well-mixed solution loaded with the compounds of interest. For hydrophobic organics (HOCs), however, such experiments are limited by the ability to measure very low-level concentration in the water. In this work we employ polymeric passive samplers to concentrate the compounds from water but the kinetics of sorption onto the passive samplers complicate the analysis of observed AC sorption kinetics. In this study, polydimethylsiloxane (PDMS) fibers were introduced to a batch experiment studying kinetics of PAHs and PCBs adsorbing in various PAC and GAC. The experiment was carried out in glass containers loaded with PCB water solution, AC, and PDMS fibers pre-loaded with performance reference compounds to define kinetics of PDMS sorption. The fibers were collected at various times and analyzed for PAHs and/or PCBs uptake mass. Assuming both the uptake of PDMS and the adsorption in AC following classic first-order behavior, a competitive sorption model was developed and closed by an analytical solution. The uptake rate constant of PDMS, the partitioning coefficients in glass, the water-AC partitioning coefficients at equilibrium, and the kinetic sorption rate constant in AC were evaluated by fitting the simulated uptake mass to experimental measurements. The derived sorption kinetics and capacity of PAC and GAC were evaluated and compared. PAC uptake is rapid with time scales typically much less than that of the PDMS while GAC kinetics were significantly slower than PAC and the PDMS and the ultimate capacity of the GAC at any finite time was substantially less than the PAC. This study demonstrated the usage of polymeric passive samplers in exploring the sorption kinetics forms of AC, even for AC whose sorption kinetics are faster than that of the passive sampler.

04.10.05 Evaluating PCB Remediation Scenarios for the Anacostia River by Linking Fate-Transport of PCB Congeners to Bioaccumulation in Aquatic Food Web

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Polychlorinated biphenyls (PCBs) are major contaminants of concern in the Anacostia River, and are responsible for fish-consumption advisories in District of Columbia (DC). Uptake of PCBs in the aquatic food web is governed by the chemical activity in surface water and sediments, which can be directly related to their freely dissolved concentration in these phases. In aquatic systems, this freely dissolved concentration is governed by mass transfer of pollutants across the air-water and sediment-water interfaces as well as direct inputs from tributaries and overflows. Differences in chemical activity govern the direction of these mass transfer processes as well as the impact of direct inputs on the receiving water body. In our previous study in the Anacostia River, measurements of surface water, sediment porewater, and gas-phase PCB concentrations using polyethylene passive samplers were able to provide the first accurate mass balance of freely dissolved PCBs in the 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, while also demonstrating that the atmosphere served as a sink for PCBs from the surface water. In the present study, parameters from an existing hydrodynamic model that simulates total freely-dissolved PCBs at a high spatial resolution 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 are being validated against measured data and model is being used to simulate impact of remediation scenarios such as natural recovery, reduction in tributary inputs, and activated carbon amendment of contaminated sediments on PCB concentrations in the Anacostia River. Model outputs will be linked to a validated aquatic food-web model to quantify impact of remediation scenarios on PCB concentrations in fish tissue, and thus used to identify the most effective remediation strategy. By focusing on a congener-level modeling approach instead of total PCBs, the predictive framework presented here can be regarded as a more effective method to utilize expensive congener-level site characterization data that is frequently necessary for baseline, risk, and performance assessments at contaminated sites, and can potentially inform the remedial decision-making process at such sites.

04.10.06 Implications of Polar Organic Chemical Integrative Sampler for High Membrane Sorption and Suitability of Polyethersulfone as a Single-Phase Sampler

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Polar organic chemical integrative sampler (POCIS) contains sorbent material enclosed between two polyethersulfone (PES) membranes. Conventionally, the sorbent acts as a sequestered medium, whereas the membrane serves only as a diffusion barrier. Currently, the sorbent uptake is correlated with water concentration using first-order kinetics, assuming negligible PES sorption. However, a significant PES uptake was reported for many contaminants, which questions the applicability of the simplified first-order kinetics. Until now, the implications of mass transfer under high PES sorption are not well-understood. This work evaluated the uptake kinetics of four high PES sorbing substituted chlorobenzenes using POCIS and single-phase PES sampler. First, laboratory calibration was performed for 60 days to assess POCIS uptake using three commonly used first-order kinetic models. Second, the suitability of PES as a single-phase passive integrative sampler was evaluated using laboratory calibration. Third, a poly-parameter liner free energy relationship (pp-LFER) was developed using published data to predict PES-water partition coefficients. POCIS calibration results demonstrated that sorbent uptake was mainly dominated by a single interaction mechanism with a linear uptake between 8 to 41 days depending on the compounds. The obtained sampling rates for the sorbent phase ranged between 0.041 and 0.054 L/day. Accumulation in POCIS membrane followed two-phase uptake kinetics, where rapid initial sorption occurred in the earlier days (< 7 days) followed by a slower matrix diffusion for longer exposure time (equilibrium not achieved), indicating membrane-controlled mass transfer. The single-phase PES sampler reached equilibrium within 19 days for all target compounds, indicating its suitability for use in the equilibrium regime. Partitioning of the target compounds with PES was determined in a range from 1.2 to 6.5 L/g. This work demonstrated that a membrane-controlled uptake was evident in POCIS for chemicals with high PES sorption, requiring a higher-order uptake model to accurately describe the mass transfer mechanisms. However, the necessity of a complex model for POCIS calibration could be avoided using a single-phase PES sampler for compounds with a higher affinity for the membrane. The developed pp-LFER model could predict the suitability of the PES sampler over POCIS for new compounds.

04.10.07 Use of Historical Data from Multiple Passive Samplers to Model Transport and Degradation of Polycyclic Aromatic Hydrocarbons in Capped Sediments

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Capping is a widespread technique to prevent the release of chemicals from contaminated sediments to surface water. Often post-construction monitoring is limited to cap physical integrity or measurements of surficial contamination immediately after construction. In this work, 4 porewater profiling sampling events over a 7 year period at 20+ locations in the Roxana Marsh area of the Grand Calumet River (East Chicago, IN) are analyzed to evaluate the fate and transport behavior of polycyclic aromatic hydrocarbons (PAHs) in a cap composed of sand and organophilic clays. A transport-reaction model was constructed from data that included (i) historical pore water concentration of PAHs as a function of depth from polymeric passive samplers, (ii) cores indicating changes in substrate as a function of depth, (iii) concentration of conservative species from equilibrium-diffusion samplers, and (iv) pore water velocity estimations. The mathematical model was simulated in CapSim software, and it describes the behavior of PAHs in a domain consisting of native sediments, the remediation cap and surface water. The main processes in the system include transport by advection and diffusion-dispersion, sorption, consolidation, deposition and degradation. The model was used to identify both key processes that have led to the PAH profile changes over time and the significance of these changes into the future. The analysis suggests that the PAHs are effectively contained by the cap as a result of sorption, new sediment deposition and degradation. Long term projections suggest that the cap should maintain a similar level of protectiveness indefinitely.

04.10.08 Impact of Hurricane Harvey on Personal Chemical Exposure

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Over 200,000 homes were damaged or destroyed when Houston, TX experienced extreme rainfall and flooding from Hurricane Harvey. Importantly, 13 Superfund sites and several chemical/petroleum facilities were involved in unplanned chemical releases into the environment due to facility shutdowns and infrastructure damage. As clean-up efforts began communities raised questions regarding the human health impact of possible increased chemical exposure resulting from the hurricane and subsequent flooding. A multi-institution team was formed to deploy personal sampling devices in the form of silicone wristbands to a longitudinal cohort of individuals (n=99) immediately after the hurricane and again one year later. The one-year post hurricane timepoint served as an estimated baseline chemical exposure for the Houston area. Using gas chromatography-mass spectroscopy, we analyzed each wristband for 1,530 chemicals which contains chemicals from multiple chemical categories including polycyclic aromatic hydrocarbons (PAH), dioxins & furans, pesticides, polychlorinated biphenyl's (PCBs), and brominated/organophosphate flame retardants. The total detections of chemicals from the wristbands from the Hurricane Harvey study were further compared to other wristband studies conducted by the Food Safety and Environmental Stewardship (FSES) laboratory within the United States in non-disaster scenarios using the same analytical method. In doing so we were able to identify chemicals/chemical categories that had hurricane driven exposure and Houston driven exposure. The mean chemicals detected/wristband was higher in both the post-flood and estimated baseline sampling periods in Houston, TX than any other comparable study. Additionally, pesticides were noted as a chemical class with Houston driven exposure since exposure frequencies were higher at both Houston, TX then in comparable studies. PAHs had a hurricane driven exposure since detection frequencies were higher only at the post flood time point. When comparing across the two time points in Houston, TX 14 of the 23 chemicals detected in 70% of the wristbands had higher concentrations post-flood, and only two had higher concentrations at the estimated baseline. When evaluating all chemicals detected at both times points it was found that PAHs and flame retardants were more likely to be found at higher concentrations post flood, and pesticides were more likely to be found at higher concentration at he estimated baseline.

04.10.09 Comparison of Nano-Graphene and Polyethylene Sheet as Passive Samplers for Detecting Organophosphate Esters (OPEs) and Implication on Field Deployment

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Organophosphate esters (OPEs) have been increasingly used as flame retardants and plasticizers. They became popular following the phasing out of brominated flame retardants (BFRs). Low cost of production and good compatibility with polymer materials made OPEs popular in various industries including textile, plastics, building materials, furniture, electronics, and vehicle parts. They are readily released into the surrounding environment by volatilization, leaching, abrasion and dissolution. Several studies connected prolonged exposure and accumulation of OPEs to various adverse health effects (immune, developmental, and neurobehavioral). OPEs have various substitutes which gives them a wide range of physical and chemical properties rendering them difficult to sample with traditional passive samplers. Only a small spectrum of these compounds was detected successfully by a single passive sampler. Polyethylene samplers (PE) have been employed as passive samplers for OPEs in the aquatic environment. We developed a novel graphene passive sampler. The pore size and thickness of the diffusion-limiting membrane of a passive sampler are the key factors in the sample selectivity. To test this effect this study proposes comparison of the two passive samplers in a controlled setting to aid the application of these samplers in field deployment. Graphene hydrogel monolith was synthesized from 10 mL of a suspension of 2 mg/mL suspension of graphene oxide and threated to yielding a cylindrical reduced graphene oxide (rGO). Polyethylene-sheets of 50 µm thickness were cut into 7 x 3cm strips and precleaned by incubation in methylene chloride and hexane for 24 h each. In the laboratory both graphene and polyethylene samplers were exposed to known mass of OPEs and tested in exposure length (1, 2, 4, 8, 16, 32 days) and different temperature and salinity conditions at 2, 10, 14, and 22 degree Celsius and salinity of 0 and 35 psu respectively. The carbon-based chemistry of graphene sampler enabled the adsorption of a wide range of contaminants with variety of substituents as oppose to the polyethylene samplers. The graphene samplers had also reached partitioning equilibrium within 72hrs of exposure with log KGH_W for individual OPEs varied between 2.44 and 4.98. The low density and large feeder pores in the structure of the formulated graphene samplers enhanced internal diffusion that partitioning equilibrium were reached very fast.

04.10.10 Development of Novel Functionalized Polymeric Thin Films for Equilibrium Passive Sampling of PFAS Compounds in Surface and Groundwater

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The demand for accurate assessment of risk to humans and ecosystems from the presence of PFAS compounds has motivated the development of passive sampling approaches. While much progress has been made in the field of passive sampling for hydrophobic organic compounds (HOCs) and metals, the field of passive sampling is still in the early stages of development for PFAS compounds. The overarching goal of this research is to develop and test a range of functionalized polymeric thin films and polymer-inclusion membranes for use in equilibrium passive sampling of PFAS compounds. To archive this goal, the following objectives have been addressed in this project: 1) development of novel polymeric thin films for equilibrium passive sampling that will be either solid or liquid sorbents embedded in a thin polymer sheet; 2) perform equilibrium partitioning studies with PFAS compounds covering a range of properties and interpret isotherm data based on sorption models; 3) test equilibrium reversibility for the top two choices of polymers and assess the use of performance reference compounds to quantify the extent of equilibrium; 4) evaluate mechanism of the sorption process. Four different materials: agarose, PDMS, CA and CTA were used to form polymer base for PFAS compounds analysis. Different parameters were optimized to determine the most suitable experiment conditions for the film formation. The potential passive sampling materials have been tested fall under 2 categories: 1) standard laboratory polymers not specifically designed for PFAS sorption, and 2) polymers with PFAS binding functional additives. A set of polymeric sheets with chosen sorbent additives have been successfully formed in the laboratory. Ongoing PFAS isotherm studies are evaluating the feasibility of the formulated thin films for use as equilibrium passive samplers. Results from the ongoing isotherm experiments and uptake kinetics experiments will be presented at the SETAC meeting.

04.10.11 Kinetic Evaluation of Isotopically Labelled Methylmercury for Use As a Performance Reference Compound in a Novel Equilibrium-Based Passive Sampler

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Mercury is a global pollutant that is transformed into the more toxic and bioaccumulative form, methylmercury (MeHg) in natural systems. Human exposure to high levels of MeHg through the consumption of contaminated fish has driven the push for adequate site assessment and remediation, necessitating accurate determination of aqueous MeHg concentrations. These concentrations, however, can be low (at pM levels in unaffected sites) and variable through time, which adversely impacts the reliability of traditional grab sampling techniques. Our novel equilibrium-based passive sampler comprised of activated carbon suspended in agarose gel (ag+AC) has provided robust and detectable MeHg concentrations in controlled, environmentally relevant settings. When deploying equilibrium-based samplers in field settings, sampler-water equilibration periods can be lengthy and difficult to predict. Performance reference compounds (PRCs) are thus employed to quantify the kinetics of exchange and deviation from equilibrium conditions. As several stable MeHg isotopes are present in nature, the presented work explores the use of a stable isotopically labelled MeHg spike for use as a PRC. Sampler-water reversible equilibrium exchange of MeHg was demonstrated in an experiment where ag+AC samplers pre-loaded with $Me^{199}Hg$ were inserted into a solution containing aqueous $Me^{198}Hg$. The uptake behavior of $Me^{198}Hg$ over time was similar to the simultaneous desorption behavior of $Me^{199}Hg$ from the pre-loaded passive samplers. This work demonstrates that a simple first-order kinetic model can describe the sampler uptake of $Me^{198}Hg$ and desorption of $Me^{199}Hg$, with comparable rate constants between the two isotopic species. Ongoing work is exploring the use of the isotopically labelled $Me^{199}Hg$ as a PRC while deploying ag+AC samplers in an environmentally relevant setting and the use of the PRC for correction of non-equilibrium conditions. Results thus far indicate promise for the applicability of an isotopically labelled MeHg spike as a PRC to verify equilibrium conditions or estimate aqueous concentrations under non-equilibrium conditions.

04.10.12 Peeping Into the Past: Lessons From Decades of Using Peepers to Measure Availability of Inorganics in Sediment Porewater

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Dialysis samplers, also called peepers, are a type of passive sampler which function by allowing sediment porewater to equilibrate with lab-provided water (peeper solution) contained in a small isolated compartment via passive diffusion through a semi-permeable membrane. After an equilibration period, the peeper is retrieved, and the peeper solution is analyzed via standard methods and reported as a concentration in water that can be easily compared to water criteria or used in fate modeling. Peepers have been used to measure the availability of inorganics at sediment sites for decades, but the lack of standard guidance documents has led to greatly varied methods, hindering the use of peepers for regulatory and decision-making purposes. To advance the maturity of peeper technology for the measurement of inorganic in porewater, we conducted a comprehensive review of the available literature to document best practices and examples of success, limitations, and unknowns associated with peeper use. Our review focused on peeper design, field handling procedures, deployment times, and post-deployment sampling processing. In our review we developed perspectives on the shortcomings and successes of peeper applications, which we synthesized into a list of best practices and gaps in methodology that can enhance peeper standardization. Several key data gaps were identified that should be addressed to advance method standardization, but also, we found several data needs for “myth busting”, that, if addressed, have the potential to simplify and optimize peeper methods. For example, our review identified fundamental knowledge gaps and method inconsistencies to limit the oxidation of geochemically sensitive compounds during post-deployment peeper sample processing and limit potential contamination of peeper solution during processing. Such gaps have resulted in a wide range of methodologies, from loosely defined to thoroughly specific, for which we will discuss the advantages and drawbacks regarding data quality and sampling effort efficiency. Special emphasis will be put on peeper processing in the field and its impact on geochemical interpretation. We will also discuss the ranges of deployment (equilibration) times for peepers, and the history of approaches to evaluate and optimize them. Lastly, we will highlight a list of critical data gaps and potential approaches for addressing them via empirical experimentation.

04.10.13 Equilibrium Porewater Measurement of PCBs and PAHs Using Direct Water Extraction and Comparison with Passive Sampling

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The freely dissolved concentration (*Cpw*) of hydrophobic pollutants in sediments is a good predictor for exposure to aquatic organisms, bioaccumulation, toxicity, and flux across interfaces. Direct extraction and measurement of sediment porewater is typically understood to be inadequate for providing a reliable measurement of *Cpw* for hydrophobic pollutants like PAHs and PCBs in sediments. Equilibrium passive sampling approaches have been developed to provide accurate measurement of *Cpw* in sediments avoiding interference from colloids and dissolved organic carbon. The objective of this research is to compare the *Cpw* of HOCs measured using passive sampling with concentrations obtained via direct water extraction method and with *Cpw* estimates from sediment concentrations and organic carbon data. Polymeric samplers made of polyethylene and polydimethylsiloxane were directly inserted into contaminated sediment *ex-situ* to measure *Cpw*. The sediment exposure was conducted in parallel experiments of active (shaking table) and static condition for 4 weeks for equilibrium. Direct measurement was performed by equilibrating the sediment with water for 1 month followed by settling and alum flocculation to remove colloids. This was followed by solvent extraction, cleanup, and analysis for PCBs and PAHs. The direct water measurement was corrected for DOC using measured DOC concentration in porewater and estimated partition coefficients for DOC. The direct water measurement with DOC correction provided consistent results with passive samplers for both PCBs and PAHs. The measurement using direct water extraction was adequate to assess narcosis toxicity of PAHs to benthic organisms that is driven by the concentrations of low to moderately hydrophobic PAHs (naphthalene to chrysene). Prediction of PCB bioaccumulation in benthic organisms agreed within 50% for all measurement methods, but it was apparent that for less contaminated sediments, the direct water extraction method would likely run into greater issues with detection limits, especially for the strongly hydrophobic PCBs. To address the challenge associated with poor detection or uncertainty of the *Cpw* measurement of the strongly hydrophobic compounds, a new extrapolation approach is proposed that can be applicable for both direct water extraction and passive sampling methods. The *Cpw* of compounds measured with a high degree of certainty are used along with the measurement of *Csed* to develop a K_{oc}-K_{ow}.

04.10.14 Identifying Regional and Local Atmospheric Sources of Organic Contaminants to the Saint Lawrence Estuary Using Passive Samplers

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Organic contaminants have been implicated in the decline in the size and health of the beluga whale population in the Saint Lawrence Estuary. Here, we present a study that seeks to identify regional and local sources potentially contributing to the atmospheric deposition of organic contaminants to the whale’s estuarine habitat. Considering the vast size and remoteness of the region, a network of passive air samplers (PASs) was identified as a viable and cost-effective approach. PASs using XAD-2 resin as a sorbent were deployed at 50 sites along the Saint Lawrence River between Montreal and Quebec City and along both the Northern and Southern shore of the Saint Lawrence Estuary for 9 months (November 2019 to August 2020). The 48 PASs that could be retrieved successfully were analyzed for polychlorinated biphenyls, organochlorine pesticides, polycyclic aromatic hydrocarbons, organophosphate esters, halogenated flame retardants and selected perfluorinated compounds. Spatial air concentration patterns revealed by the PAS are highly divergent for different compound groups and are indicative of different types of atmospheric emissions. For example, polychlorinated biphenyl concentrations were generally elevated along the Montreal-Quebec City corridor, consistent with the relatively higher population density and industrial history of these areas. Superimposed on these large-scale regional concentration gradients, the samplers also identified potential emissions sources of local importance, such as an incinerator in Quebec City or the industrial facilities in Sept-Îles. On the other hand, for some of the quantified compounds, the spatial concentration patterns indicate that the estuary is a source of contaminants to the local atmosphere rather than the other way around. For example, air concentrations of the pesticide α-hexachlorocyclohexane and the natural halogenated compound tribromo-anisol were elevated along the outer estuary, especially on the south shore. Such a pattern is consistent with the prevailing northwesterly winds blowing compounds volatilizing from the marine environment to the samplers deployed along the shores.

04.10.15 Development and Application of a Passive Sampler (POCIS) for Monitoring Per- and Polyfluoroalkyl Substances (PFASs) in a Drinking Water Treatment Plant

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Passive sampling has been known as one of the monitoring methods because it overcomes low detection limits of micropollutants in the environment and presents a representative concentration value through long-term monitoring. Among various micropollutants, per- and polyfluoroalkyl substances (PFASs) have been often detected in drinking water and potentially have high risks to humans and environment at trace levels. PFASs are currently being studied worldwide due to its persistent and recalcitrant in water. In this study, a passive sampler, polar organic compound integrative sampler (POCIS), is applied to monitor 3 PFASs (perfluorooctanesulfonic acid, perfluorooctanoic acid, and perfluorohexanesulphonic acid; PFOS, PFOA, and PFHxS) in a drinking water treatment plant. Before applying a POCIS in a drinking water treatment plant, three steps are needed. Firstly, sampling rates for each compound are derived through the experiment in a laboratory. The second step is to confirm whether the sampling rate is accurate under the concentration fluctuations. The concentration change scenarios are done as three different concentration changes for 10 days in the laboratory to confirm whether it is calculating the exact time-weighted average concentration (C_{TWA}) from the derived sampling rate. At last, a cage is designed to protect the sampler from hydrological effects, thereby verifying reliability to minimize effects caused by a flow fluctuation. It is anticipated that the passive sampling contributes to managing the risk of PFASs to human and the drinking water treatment plant economically.

04.10.16 Exploring Personal Chemical Exposure Profiles of Vulnerable Populations in Emerging Economies Using State of the Art Passive Sampling, Instrumentation, and Software

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Individuals in emerging economies face numerous chemical exposures; these include emissions from rapidly growing industries lacking the infrastructure, investment, and enforcement to reduce harmful contaminants in emissions. Furthermore, daily activities using low-cost alternatives for heat, fuel, food, pest control, and sanitation/refuse disposal among poorer communities also leads to unique exposure profiles. Only recently has technology begun to emerge to comprehensively capture personal exposure profiles to thousands of chemicals. For this purpose, we developed the FreshAir wristbands which encase polydimethylsiloxane which passively uptakes a wide range of volatile and semi-volatile chemicals. Furthermore, we introduce in this talk new software for rapidly assigning confidence to suspect screening datasets (screening nearly 1 million chemicals). We incorporate these annotations into a workflow for determining those compounds most likely to be of health concern using the EPA's hazardous comparison dashboard and CompTox Dashboard. For the first time we implement this technology across various emerging economies including China, India, and South Africa, showing alarming exposures which greatly differ across region, season, and depending on behaviors and activities. For example, insecticides used for malaria control differed across countries, with dichlorvos measured across almost all of the older population studied in Jinan, China, whereas DDT, propoxur, piperonyl butoxide, and other insecticides were measure across over 75% of the young children studied in South Africa. Most insecticides were seen to drastically increase during wet warm seasons when mosquito populations were highest. A diverse range of combustion products including furans and PAHs were found in both India and South Africa, where refuse is burned for disposal and both biomass and refuse is burned for cooking. These findings provide us a first glance of chemicals of concern in vulnerable populations such as children and the elderly, which should be investigated further to reduce any harmful exposures in these regions.

04.10.17 Comprehensive Review of Passive Sampling to Evaluate Sediment Remediation Efficacy

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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 emerged as 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. In some instances, because PSD data is shown to correlate with contaminant bioavailability, it may be a more powerful tool for decision-making than conventional bulk sediment measurements. This study comprehensively reviewed literature on PSD use during the sediment remediation process, from feasibility studies to monitoring remedy effectiveness. Authors of over 100 articles, reports, and conference presentations have used PSDs to support sediment remediation projects at 57 sites in the US, Europe, and Asia. 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, highlighting the advances and growing acceptance of polymeric PSDs. 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 range of findings, in some cases, PSD data indicated the remedial action selected did not result in the desired effect, whereas conventional bulk sediment sampling indicated the remedial action was successful. This review also noted several key areas for improvement that could lead to greater acceptance and use of PSDs in the sediment remediation process: (1) more comparison between in-situ and ex-situ passive sampling for sediment porewater, (2) continuing to standardize analytical, field, and data analysis methods, (3) clarification of the effect of black carbon on the interpretation of in-situ PSD data, (4) research into effects of non-aqueous phase liquids (NAPLs) on PSDs, and (5) development and standardization of PSDs for more hydrophilic organic contaminants often associated with sediments (e.g., munitions, polar pesticides, and some per- and polyfluoroalkyl substances (PFAS)).

04.10.18 Development of an Equilibrium-Based Passive Sampler to Simultaneously Predict Porewater Concentrations of Arsenic, Cadmium, and Mercury

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Arsenic, Cadmium, and Mercury are toxic heavy metals that are commonly co-present in environmental systems. Humans are exposed to these metals through contamination of groundwater, fish, and vegetation. Aqueous concentrations in groundwater, porewater, and surface waters are key measurements for site assessment, however, they can be low and temporally variable. This work presents the development of a novel equilibrium-based polymeric passive sampler for the simultaneous measurement of these toxic metals. Samplers synthesized by casting a sorbent (activated carbon or thiol-SAMMS) within an agarose gel were inserted in concentration-dependent soil slurry isotherms. Measured porewater As, Cd, and Hg concentrations were positively correlated with the corresponding sampler concentrations for both the AC-based sampler (ag+AC) and the thiol-SAMMS sampler (ag+SAMMS). Both samplers were also deployed *in situ* in several rice paddies at the University of Delaware, after which the sampler and surrounding porewater concentrations were measured for As, Cd, and Hg. As concentrations in both ag+AC and ag+SAMMS samplers positively correlated with corresponding porewater concentrations. Additionally, samplers exhibited good reproducibility of As and Cd among replicates. To evaluate their predicting power, partitioning coefficients were calculated using the sampler and porewater concentrations in the soil slurry isotherm, then were used to estimate the corresponding porewater concentrations in the rice paddies from the sampler concentrations. The ag+AC sampler predicted As and Hg porewater concentrations (from a single grab sample) within a factor of two, while the ag+SAMMS samplers provided predictions for As and Cd within a factor of 2.5. This work demonstrates the capability for the ag+AC and ag+SAMMS samplers to produce simultaneous and reproduceable measurements of As, Cd, and Hg with robust predicting power.

04.10.19 Evaluation the Effectiveness of Multiple Sampling Devices for Use in Contaminant Monitoring of Marine Waters

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Environmental sampling can often be challenging, particularly in coastal ecosystems where tides, episodic surface runoff and environmental accidents can influence water concentrations of organic contaminants. Generally, discreet water sampling can offer a direct measure of chemicals at a given point in time, but only through intensive and repeated sampling can this effort lead to an integrated estimate for concentration ranges representative of a location. Alternatives to discreet sampling are passive in situ integrative sampling devices which are designed to adsorb water borne chemicals over time. More recently, devices that link active and passive sampling have become available to researcher. In this study, three organic chemicals were used as surrogates for multiple classes of contaminants (bifenthrin, pyrene and triclosan) to track sampling device effectiveness relative to discreet sampling, in an estuarine mesocosm system. Over a 28 day exposure, samplers were deployed at "low" and "high" concentrations. Samplers included two passive (POCIS, silicone bands) and one active (CLAM) devices. Using published rate constants, POCIS and silicone band concentrations were estimated and resulting concentrations were determined in order to evaluate strengths of each sampling device.

04.10.20 Evaluating Sedimentary PAH Bioavailability Based on Equilibrium Partitioning and Passive Sampling at the Dover Gas Light Superfund Site (Dover, Delaware, USA)

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From 1859 to 1948, the Dover Gas Light plant produced combustible gas for industrial, commercial, and residential applications using pine resin, coking coal, oil and wood, and finally, a coal-gas process. Waste coal tar was discharged to the Saint Jones River in Dover (Delaware, USA) via a ditch and culvert and, following plant closure in the 1940s, through groundwater flow from buried structures on the site. By the end of twentieth century, PAH contamination of the sediments in the Saint Jones River was suspected to have occurred and state and federal agencies initiated environmental assessments of the newly designated Superfund site. The current study investigated the spatial distributions of total PAHs in Saint Jones River sediments adjacent to the site and evaluated their bioavailability. In 2017, 34 sediment cores were collected, sectioned, and analyzed using an on-site fluorometric screening technology indicating total PAH sediment concentrations ranging from 0.1 to 15,000 mg/Kg (wet). A subset of cores involving 20 samples of various depths were selected and further analyzed by conventional GC/MS analysis for 16 parent PAHs. In addition, a 34 day *in situ* deployment of polyethylene passive samplers was performed to measure vertical bioavailability profiles of parent PAHs in sediments at three locations and overlying waters at four stations. Freely dissolved concentrations (C_{free}) of total PAHs were estimated based on equilibrium partitioning (EqP) of the GC/MS results and the passive sampling findings. C_{free} values were used to calculate acute and chronic toxic units ranging from 1.4 to 56 based on EqP and 1.3 to 15 based on passive sampling. For six samples where comparative data were available, EqP calculations over-estimated bioavailability by < 2 to 54-fold. Combining rapid field measurements with more accurate analyses of sediment concentrations and bioavailability in a tiered framework allowed for a time-efficient and cost-effective site investigation.

04.10.21 Misbehaving Passive Sampler Performance Reference Compounds and How to Handle Them

B.G. Pautler, J. Roberts, SiREM; A.P. Wang, Geosyntec Consultants, Inc. / Civil & Environmental Engineering; C.L. Thomas, J.M. Thompson, J.M. Conder, Geosyntec Consultants

Passive sampling devices (PSDs) have been shown to present many advantages over conventional sampling methods for quantifying the availability of hydrophobic organic compounds (HOC) in sediment, soil, surface water and stormwater. PSDs can provide data to estimate contaminant bioavailability and toxicity to environmental receptors that are more accurate than conventional grab or mechanically extracted samples, as they quantify the freely dissolved contaminant concentrations (C_{free}). Approaches that rely on using Performance Reference Compounds (PRCs) added to PSDs prior to deployment are standard practice for short-term deployments in which equilibrium concentrations in the PSDs are not achieved. Despite the rigorous application of standardized sampler preparation and analysis protocols, PRC anomalies have been observed by multiple investigators at sites of different hydrodynamic conditions, while using various types of PSDs, and various classes of PRCs. PRC anomalies occur when the concentration of a PRC in a PSD after retrieval from a field exposure is much higher or lower than is expected given the hydrophobicity of the PRC and/or the behavior or other PRCs used in the same device or blank. Such anomalies represent outliers with respect to other PRC data from the same device, typically resulting in exclusion of the data which leads to increased error and uncertainty associated with estimation of C_{free} values. In severe cases, all PRC data from a deployed sample may be anomalous, preventing the estimation of C_{free} values. In this presentation, we will review representative PSD datasets containing anomalous deuterated PAH and rare PCB congener PRC anomalies. The evaluated datasets included tidal and non-tidal systems, *in situ* and *ex situ* deployments, and sediment and surface water matrices. These results showed that PRC desorption anomalies are neither site specific, PRC specific, laboratory specific, nor sampler material specific. In addition, we will present a pragmatic mathematical approach for using anomalous PRC data to calculate C_{free} values.

04.10.22 Measurement of Total Microcystins Using Integrative Passive Sampling and Gas Chromatography-Mass Spectrometry Analysis

R. Grewe, J.B. Belden, Oklahoma State University / Integrative Biology

Harmful algal blooms can occur in water bodies receiving excess nutrients, and sometimes lead to the production of cyanotoxins, including microcystins, the most common freshwater cyanotoxins. Microcystins present a risk to both terrestrial and aquatic wildlife at low concentrations, and their production fluctuates heavily with environmental parameters. Therefore, developing sensitive methods for detecting microcystins is important for monitoring potentially contaminated water bodies. However, with over 100 variants, detecting total microcystins in water samples can prove difficult. Oxidizing and cleaving the 2-methoxy-3-methyl-4-phenylbutyric acid (MMPB) molecule found in all microcystin molecules for analysis allows for the detection of total microcystins in samples without the use of multiple reference standards. Employing passive sampling techniques can further assist the detection of microcystins in water bodies with fluctuating production or low concentrations through the continuous collection of the analyte. This study investigates a new method for gas chromatography-mass spectrometry (GC/MS) analysis of MMPB (and thus total microcystins) collected via passive sampling. Polar organic compound integrative samplers will be employed along with a new method for derivatizing MMPB for GC/MS analysis using N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA). Additionally, the possibility of cleaving the MMPB molecule while still sorbed to the passive sampler will be investigated to potentially improve upon the time-consuming extraction step in passive sampling. Coupling the analysis of total microcystins via the MMPB molecule with passive sampling can provide a more simplified and comprehensive analysis microcystins in contaminated water bodies.

04.10.23 Passive Sampling of Per- and Polyfluoroalkyl Substances (PFAS) Using Pine Needles and Multidimensional Measurements

K. Kirkwood, North Carolina State University / Chemistry; S.M. Belcher, North Carolina State University / Biological Sciences; E.S. Baker, North Carolina State University / Department of Chemistry

Per- and polyfluoroalkyl substances (PFAS) are a class of manmade organofluorine chemicals used in a variety of household and industrial applications. PFAS have become a global concern due to their environmental persistence, bioaccumulative nature, and associations with adverse health effects, thus monitoring both spatial and temporal PFAS presence is crucial. PFAS levels are commonly monitored in surface and groundwater, soil, and wildlife samples, however assessing atmospheric PFAS is less common due to the necessary expensive equipment which can only be placed at a limited number of sites. Recently, the passive sampling capabilities of trees have been investigated to address this challenge. For example, pine needles possess highly adsorptive wax cuticles and have been effective passive samplers for a wide variety of environmental contaminants, including PFAS compounds with long aliphatic chains known as legacy PFAS. To understand PFAS contamination in North Carolina from point sources such as a fluorochemical manufacturer, military bases, and airports, we leveraged the passive sampling capabilities of pine needles. The PFAS in the needles were extracted with an optimized protocol and analyzed using a non-targeted platform coupling liquid chromatography, ion mobility spectrometry and mass spectrometry (LC-IMS-MS) separations, allowing simultaneous hydrophobicity, size, and mass evaluations. This new method resulted in the identification of over 60 PFAS compounds in the pine needles, along with the detection of more than 10 unknown PFAS species. Furthermore, the detected species covered all of the legacy compounds identified in previous studies, as well as multiple classes of emerging PFAS with shorter aliphatic chains and structural modifications such as ether linkages and branched or cyclic aliphatic chains, not previously detected in pine needles. This method was also successful in identifying multiple PFAS point sources, as well as longitudinally monitoring the levels of the diverse PFAS compounds across North Carolina to address environmental concentration changes due to introduction and remediation efforts. Additionally, archived pine needles were analyzed to assess historical PFAS levels in North Carolina.

04.10.24 Development and Validation of Two Polymers for In Situ Passive Sampling of Munitions Compounds

E. Shipley, P. Vlahos, University of Connecticut / Departments of Marine Sciences and Chemistry; R.M. Burgess, U.S. Environmental Protection Agency / Atlantic Coastal Environmental Sciences Division (ACESD)

Unregulated dumping of unexploded ordnance into coastal waters over the last century has raised questions about the identification and quantification of munitions compounds such as 2,4,6-trinitrotoluene (TNT) and its derivatives in various environmental mediums. Though typically present at low concentrations in seawater and sediment, many of these compounds have known human health risks and potential ecological impacts. Given the low concentrations, passive sampling is a promising avenue of research providing more environmentally-relevant data on these compounds in an economical and low maintenance way. This work examines two promising polymers, ethylene vinyl acetate (EVA) and polyoxymethylene (POM), for their utility toward detection and quantification of munition freely dissolved concentrations (C_{free}). The C_{free} was selected as it provides a good surrogate for the bioavailable concentration of the munitions in marine systems. Experiments utilize natural seawater and sediments retrieved from coastal areas affected by munition usage and disposal to determine munition C_{free} in sediment porewater, sediments, and overlying water using a mass balance approach. Laboratory results have shown uptake of several munitions compounds by each sampler polymer in both seawater and sediment slurry systems. Samplers have also been validated *in situ* in one field study in Ostrich Bay, Washington. These samplers will provide a low-cost technique to inform environmental remediation and monitoring programs at military sites and any facility where munitions compounds may be of concern.

04.10.25 Calibrating a Passive Air Sampler for Semi-Volatile Organic Compounds Using Continuous Active Air Sampling and Long Deployment Periods

Y. Li, F. Zhan, University of Toronto, Scarborough / Physical and Environmental Sciences; Y. Lei, University of Toronto / Physical and Environmental Sciences; C. Shunthirasingham, H. Hung, Environment and Climate Change Canada / Air Quality Processes Research Section; F. Wania, University of Toronto, Scarborough / Physical and Environmental Sciences

The use of passive air samplers (PAS) for atmospheric semi-volatile organic compounds (SVOCs) has expanded over the past decades. Confident use of a PAS requires knowledge of its uptake kinetics in their dependence on meteorological conditions and chemical properties and of the limits of linear uptake of more volatile SVOCs. Such knowledge is gained through calibration studies involving the side-by-side deployment of active and passive air sampling techniques. Here we describe a calibration of the XAD-PAS, which uses styrene-divinylbenzene-copolymeric resin as a sorbent. The resin's high capacity increases the likelihood of linear uptake, including for relatively volatile SVOCs and during long deployments. Several elements of this study aim to improve on earlier calibration studies, by (i) being located in an urban area (campus of the University of Toronto Scarborough), where air concentrations should be sufficiently high to facilitate reliable quantification of many SVOCs, (ii) lasting long enough for the XAD-PAS to potentially exceed the linear uptake period of more volatile SVOCs (one full year, June 2020 to June 2021), (iii) including a large number of PAS deployments (12 retrieval dates spaced 4 weeks apart, all duplicated), (iv) involving continuous, rather than episodic, active air sampling using flow rates (mid-volume pump) and sampling times (1 week) that seek a balance between minimizing breakthrough losses and collecting sufficient amounts for reliable quantification, (v) collecting SVOCs in the gas and particle phase separately, and (vi) aiming for quantification of a large number of SVOCs by both targeted and untargeted analytical approaches. It is hoped that this "gold standard" calibration experiment will succeed in greatly expanding the knowledge base on sampling rate and length of linear uptake for a great variety of both legacy and emerging SVOCs in the XAD-PAS.

04.10.26 Polar Organic Chemical Integrative Sampler for Compound Specific Isotope Analysis of Substituted Chlorobenzenes at sub-µg/L Concentrations

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Compound specific isotope analysis (CSIA) is an established tool to demonstrate *in situ* degradation of traditional groundwater contaminants. To date, CSIA is mainly limited to heavily contaminated sites due to the high detection limit of isotope ratio mass spectrometers. Recently, CSIA has been reported for µg/L to higher ng/L concentrations by extracting 10 to 100 L of water using solid-phase extraction (SPE), which is laborious and often resulted in significant method-induced isotope fractionation. Thus, an efficient preconcentration technique is necessary to expand the application of CSIA to trace contaminants. This work evaluated the compatibility of polar organic chemical integrative sampler (POCIS) with CSIA for substituted chloronitrobenzenes, chloroanilines, and nitrotoluenes at low µg/L concentrations. First, we evaluated whether POCIS can accumulate enough target compounds for CSIA. Second, method-induced isotope fractionation was evaluated using 60-day laboratory kinetic experiments. Third, the performance of the POCIS-CSIA method was assessed in pilot constructed wetlands for the same deployment time. The results demonstrated that neither diffusion towards and through the membrane and sorbent nor adsorption onto the membrane and sorbent phases resulted in significant changes in analytes' carbon isotope signatures (< 0.7‰), except for 3,4-dichloronitrobenzene (1.6‰). Results also suggested that one POCIS alone can accumulate the target analytes for CSIA at concentrations down to 1-10 µg/L, which would require up to 10 L of water extraction using SPE. Moreover, POCIS could potentially be used for CSIA at ng/L range when multiple POCIS are combined, or additional sorbent mass is added to the sampler. Negligible membrane biofouling of the field deployed POCIS was observed by scanning electron microscopy and DNA analysis, thus indicating an insignificant effect of biofouling on the obtained field isotope signatures of the compounds. This work opens a new avenue for CSIA in low concentration environments, e.g., rivers, wastewater treatment plants, constructed wetlands, and long-term remediation sites without extracting large volumes of water. Because POCIS is specifically designed for polar compounds, this work also enables the application of CSIA to the thousands of polar emerging contaminants present in the environment, such as pesticides, pharmaceuticals, and flame-retardants.

04.10.27 Effects of Dissolved Organic Carbon on Zinc Toxicity to Embryos of S. Purpuratus and M. Galloprovincialis as Measured by Diffusive Gradient in Thin- Films

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Dissolved zinc concentrations used in marine water quality criteria do not necessarily reflect the bioavailable fraction for many organisms. Diffusive gradients in thin-films (DGT) passive sampling devices potentially better quantify bioavailable zinc than grab-sampling in seawater, as DGT devices accumulate only the labile fraction. Laboratory exposure of LSNP-NM DGT devices and *S. purpuratus* and *M. galloprovincialis* embryos in seawater with varying concentrations of Zn as well as varying characteristics and concentration of dissolved organic matter was conducted to determine the extent to which DGT adsorption of Zn mimics toxicity buffering in coastal marine environments. Results provide preliminary median effective concentration (EC50) ranges of 47.7-74.6 ug/L as C_{DGT} Zn for *S. purpuratus* and 89.9-135 ug/L as C_{DGT} Zn for *M. galloprovincialis* over the range of 0.789-1.91 mg/L dissolved organic carbon (DOC). Toxicity buffering by DOC was observed, suggesting that site-specific zinc thresholds in seawater would be suitable.

04.10.29 Graphical Tools for the Planning and Interpretation of Polyurethane Foam Based Passive Air Sampling Campaigns

Y. Li, University of Toronto, Scarborough / Physical and Environmental Sciences; J.M. Armitage, AES Armitage Environmental Sciences, Inc; F. Wania, University of Toronto, Scarborough / Physical and Environmental Sciences

Due to low cost and easy handling during sampling and extraction, passive air samplers (PASs) using polyurethane foam (PUF) as a sorbent have become the most commonly deployed PASs for semi-volatile organic compounds (SVOCs). However, because of the relatively low sorptive capacity of PUF for many SVOCs, PUF-PAS are generally not operating in the linear uptake phase, which implies the need to consider how temperature, wind speed, deployment length and chemical properties interact to determine the amount of a target chemical taken up and the fraction of a depuration compound (DC) being lost during deployment. Guidance is therefore necessary to quantitatively interpret curvi-linear uptake in the PUF-PAS and avoid selection of DCs unsuited to the deployment conditions. While existing numerical models simulating these processes can provide such guidance, they have not been widely adopted. In this study, the PAS-SIM model and theoretical calculations are used to generate graphical tools, that make simulation results accessible without the need to run the model. Specifically, we generated five charts that display (i) the inherent sampling rate as a function of wind speed and a chemical's molecular diffusivity, (ii) the length of the linear uptake period as a function of chemical properties, temperature and deployment length, (iii) the time to 95 % equilibrium as influenced by chemical properties, temperature and wind speed, (iv) the fractional loss of DCs in its dependence on chemical properties, temperature and deployment length, and (v) the influence of chemical properties, temperature and the total suspended particle concentration on the extent of sorption to atmospheric particles. The charts also include information that allow for the assessment of the influence of parameter uncertainty. It is hoped that these charts lower the hurdles to planning and interpreting sampling campaigns based on a mechanistic and quantitative understanding of how the PUF-PAS functions.

04.10.30 Detecting Organic Contaminants in the Marine Environment Using Silicone Band Sampler Technology

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The use of passive samplers as a tool in the environment to determine the presence of organic contaminants in aquatic environments has been around for a number of years. The use of small, inexpensive silicone bands however is relatively new compared to other passive sampler technology. Researchers looking for an affordable, inexpensive way to conduct monitoring efforts have turned to this technology. Although literature exists on research conducted in fresh water aquatic environments, there is little information recording the use of silicone bands in the marine environment. In this study, we examine annual seasonal patterns of organic contaminants targeting polycyclic aromatic hydrocarbons, pyrethroids, and fipronil at 15 sites in and around the Charleston, SC coastal waters. These sites were chosen as they are paired with sites currently monitored by community resources (i.e. Charleston Waterkeeper) for coliform bacteria and general water quality with the objective to determine potential differences among sites and between seasons. The design includes deployment of bands for 28 days each season for a year. Preliminary results, including Fall 2020 and Winter 2021 deployments, suggest that silicone bands can be used to detect measurable amounts of organic contaminants, and that when making comparisons between seasons and field sites as well as between compound classes, noticeable differences are present. By pairing with community interest groups, a larger body of information can be acquired and factors such as salinity and tide that may affect band performance can be evaluated.

04.11 Passive Sampling: Solutions to Sampling Across Environmental Compartments

04.11.02 Quantifying Optimal Conditions for Equilibrium Passive Sampling in Groundwater Monitoring Wells

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Passive sampling devices (PSDs) have been shown to present many advantages over conventional sampling methods for quantifying the availability of hydrophobic organic compounds (HOC) in sediment, soil, surface water and stormwater. PSDs can provide data to estimate contaminant bioavailability and toxicity to environmental receptors that are more accurate than conventional grab or mechanically extracted samples, as they quantify the freely dissolved contaminant concentrations (C_{free}). Approaches that rely on using Performance Reference Compounds (PRCs) added to PSDs prior to deployment are standard practice for short-term deployments in which equilibrium concentrations in the PSDs are not achieved in the deployed environment. C_{free} determination using PSDs have drawn interest from hydrogeologists due to its advantages compared to traditional active low-flow pumping of groundwater. To explore the applicability of PSDs for Cfree determination of subsurface contamination, PSDs were deployed in groundwater monitoring wells situated in a variety of hydrogeological conditions. PSDs deployed in wells with low water exchange/flow (e.g.,0.15 ft/day) exhibited PRC anomalies, suggesting a negative PRC depletion rate, a highly unlikely phenomenon. This is likely due to unfavorable (slow) thermodynamic equilibration between the water and samplers, as well as the low well volume, that limits the mass of PRCs that diffuses out of the samplers (into the water) during deployment. In contrast, PSDs deployed in groundwater monitoring wells in a fast-moving aquifer (2,000 – 3,000 ft/day) revealed favorable equilibration that is comparable to conditions in aquatic systems (surface water and sediment). These results will be used to outline the best working conditions for the application of PSDs to measure groundwater C_{free}.

04.11.03 Sediment Passive Sampling Data Accurately Predicts Concentrations in Benthic Invertebrate Tissue

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Measurements of the concentrations of freely-dissolved (Cfree) PCBs produced with passive sampling in aquatic sediment relate well to concentrations of PCB benthic organisms. To evaluate the ability of Cfree measurements to predict concentrations in tissue, we applied generic model-derived Bioconcentration Factors (BCFs) to Cfree measurements from core samples obtained from a pilot study involving thin sand Enhanced Natural Recovery (with and without Activated Carbon) in the Lower Duwamish Waterway (Seattle, WA, USA). Cores were used in a 28-day laboratory bioaccumulation study with polychaete (Nephtys caecoides) and bivalve clams (Mya arenaria); Solid Phase Microextraction (SPME) passive samplers were added to the cores alongside organisms to measure Cfree PCBs. Individual PCB congeners were measured in the SPME and tissues (3 composite samples from each of the two areas). Cfree results calculated from the analysis of PCBs in SPMEs were then multiplied by BCFs from a bioaccumulation model (Arnot and Gobas, 2003) to predict concentrations of each congener in polychaetes and clams. Model-generated BCFs for clams were multiplied by an adjustment factor of 0.5 to account for the fact that a portion of the clam PCB exposure was to overlying water. Cfree-predicted concentrations of PCB congeners in polychaetes were within a factor of 3 of measured concentrations for 83% of the 699 detected congener measurements. Total concentrations of PCBs in polychaetes ranged from 17 to 61 ng/g, wet weight (ww), whereas Cfree-predicted concentrations ranged from 19 to 115 ng/g, ww. All polychaete tissue predictions for total PCBs were within a factor of 2 of measured values. Cfree-predicted concentrations of PCB congeners in clams were within a factor of 3 of measured concentrations for 92% of the 675 detected congener measurements. Total concentration of PCBs in clams ranged from 3.9 to 23 ng/g, ww, whereas Cfree-predicted concentrations ranged from 3.2 to 20 ng/g, ww. All clam tissue predictions for total PCBs were within a factor of 2 of measured values. Compared to approaches with measurements of PCBs in bulk sediment, results suggest that the combination of Cfree measurements and bioaccumulation modeling can provide relatively accurate predictions of concentrations of PCBs in sediment-dwelling invertebrates. This modeling approach may facilitate the use of passive sampling results in ecological and human health risk-based decision-making applications.

04.11.04 Using Sediment and Water Column Passive Sampling and Transport Modeling to Assess PCB Mass Balance in an Estuary

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Polychlorinated biphenyls (PCBs) are a class of legacy pollutants that drive health risk at many contaminated sediment sites. Remedial actions typically seek to reduce risk by removing or sequestering sediments with concentrations exceeding risk-based thresholds. Successful remedy design depends on correctly identifying the sources controlling water column and sediment concentrations, but these may not be effectively captured by sediment sampling alone in the event of ongoing external inputs (e.g., upstream inflows, point sources like combined sewer overflows, groundwater discharges) or unidentified sediment flux hotspots. To aid such efforts and help inform decision making, we investigated a coordinated use of sediment and water column passive sampler measurements and modeling to evaluate the mass balance of dissolved PCBs in an urban estuary, the Lower Duwamish Waterway near Seattle, USA. Polyethylene passive samplers were used to measure freely dissolved PCB concentrations in porewater and bottom water (co-located across the sediment-water interface) at locations throughout the estuary. The measured chemical gradients were used together with estimates of diffusive bottom boundary layer thickness from acoustic doppler current profiling to estimate sediment-water fluxes of dissolved and colloid-bound PCBs due to diffusion and bio-irrigation. The mass balance of dissolved PCBs in the estuary due to these fluxes alone was characterized by using them as input to a simulation of tracer transport with a three-dimensional hydrodynamic model, i.e., assuming that tidal flushing is the sole sink of PCBs. Comparing the predicted water column concentrations with measurements indicated that the diffusive fluxes from the sediments were too small to explain the measured water column concentrations. The results suggest that either other bed-to-water transfer mechanisms or other inputs must be predominant.The mass balance evaluations were augmented with inverse modeling to infer the source locations that would be consistent with water column PCB measurements. The hydrodynamic model was used to simulate concentrations generated by unit tracer input at potential source locations and the source strengths that yielded the best match to the measured concentrations were calculated.

04.11.05 Quantifying Tree Exposure to Particulate Matter Using Passive Samplers

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More than half of the world's population currently lives in urban areas, and this proportion is expected to rise to 60% by 2030. Past research has shown that urbanization leads to fragmentation of forested ecosystems, increasing the abundance of trees located at the forest "edge" and exposing them to greater concentrations of air pollutants than trees in the forest interior. It is well documented that anthropogenic air pollution, such as particulate matter (PM; from sources like road dust or vehicle emissions), in urban areas harms human health through detrimental effects on respiratory and cardiovascular systems. One strategy for PM mitigation is planting trees in cities, but studies show deposition of PM onto leaves harms plants. However, remote sampling of PM has proven to be challenging due to PM sampling mainly being conducted using active samplers. Due to the negative impact of high PM concentrations on human and vegetation health, we used UNC-PAS (University of North Carolina-Passive Aerosol Samplers) to quantify the potential tree exposure to different quantities and compositions of PM in forest edges (against a roadway or field) and interiors (90 m from nearest edge) in eight sites along an urbanization gradient that spanned urban Boston to rural Petersham, MA. Passive samplers were attached to two oak trees at the forest edge and two oak trees at the forest interior to represent ambient concentrations and elemental compositions of PM. Additionally, leaves from each tree were rinsed and analyzed for concentrations and elemental composition of insoluble PM were determined. Comparisons between PM on passive samplers and PM on leaves from all trees were made to determine if the passive samplers represent the quantities and compositions of PM on leaves. Leaves were also examined for damage through stomata clogging, trichome deformation, and leaf wax desiccation due to PM exposure and to determine potential relationships between quantity, size, and elemental composition of PM and leaf damage. This work demonstrates the use of passive samplers as tools to address the challenges of assessing ambient PM concentrations and exposure of trees to this pollution type.

04.11.06 Application of Passive Samplers to Support Risk Assessment and Long-Term Monitoring

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Developing cleanup levels for sediment remediation and monitoring at aquatic sediment sites often requires an estimate of tissue concentrations in aquatic organisms. However, the current approaches of collecting field tissues or conducting laboratory bioaccumulation tests have inherent uncertainties and can be time and cost intensive. Literature based biota-sediment models used to predict tissue concentrations from sediment concentrations can lead to inaccurate and overly conservative estimates of risk. Passive samplers that measure contaminant concentrations in the porewater (Cfree) provide a useful and cost-effective tool to predict tissue concentrations at sites where tissue collection and bioaccumulation testing are difficult or where a higher frequency or density of tissue concentration estimates are needed. Previous ESTCP and SERDP efforts have provided significant resources to develop passive sampling methods to measure Cfree and their ability to predict tissue concentrations. Similarly, methods for using risk assessment and probabilistic models are routinely used with tissue data to determine risk based contaminant thresholds. However, in some cases it may be appropriate to use passive-sampler-derived exposure concentrations as input to support risk models and monitoring. The primary objective of this project is to demonstrate an approach to develop site-specific bioaccumulation Cfree models to enable more cost effective risk informed decision-making at contaminated sediment sites. Bioaccumulation models will be developed using field and laboratory exposures of deposit- and suspension-feeding benthos concurrent with deployment of solid-phase microextraction (SPME) passive samplers. Benthic organisms are being exposed concurrently with colocated SPME samplers in both the field and laboratory for both a freshwater and marine sediment site. Contaminant concentrations measured in porewater and tissues are then being used to derive biota-porewater accumulation factors as well as site-specific regression models which can then be applied in probabilistic risk assessments. The use of site-specific regression models may allow for the option of using of passive samplers to augment more expensive tissue analysis of resident infauna and/or bioaccumulation testing for assessing risks and long-term monitoring.

04.11.07 Source Tracking of PCB Contamination Using Passive Samplers: The Baltimore Story

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Polychlorinated biphenyls (PCBs) are major contaminants of concern in the Baltimore region. Several PCB total maximum daily loads (TMDL) were developed in the area to decrease PCB loads and restore water quality standards that are protective for the designated use of the waterbody, i.e. "fishing" as well as "marine and estuarine aquatic life and shellfish harvesting" in the Baltimore region. One major challenge for TMDL implementation is the identification of PCB sources that are contributing to the elevated concentration in fish and shellfish. In collaboration with the City of Baltimore, Baltimore County, Anne Arundel County, Maryland Department of Environment (MDE), and U.S. Geological Survey (USGS), we monitored freely dissolved PCB concentrations, as it is indicative of biological uptake into aquatic organisms, and performed a time integrated measurement over 1-3 months to get better representation of PCB concentrations that aquatic life are exposed to. We deployed low density polyethylene (LDPE) sheets in two segments of the Tidal Chesapeake Bay with approved TMDLs, i.e. Curtis Bay and Back River, and within several tributaries and sub-tributaries to identify the ongoing source(s) of contamination of the segments. Additional monitoring was performed in the effluent of the Back River wastewater treatment (BRWWTP) and then tracked back at few locations within the sewer lines of Baltimore City. Freely dissolved PCB loadings from tributaries and BRWWTP were calculated and compared to identify the major sources of PCB, which may be leading to the elevated concentrations detected in the aquatic life. Preliminary results showed that the BRWWTP was a major contributor to the freely dissolved concentrations inputs into Back River, with a potential origin from the old Fat Oil and Grease (FOG) present in Baltimore City sewer lines.

04.11.08 Bioaccumulation of Pollutants and TMFs in a Pristine Lake: Multimedia Passive Equilibrium Sampling in Aquatic Ecosystems

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Passive equilibrium samplers (PESs), based on polymers such as silicone, offer a superior approach to assess bioaccumulation of pollutants compared with exhaustive extraction methods, since they avoid the potential bias resulting from normalization of the total concentrations of chemicals to the main sorptive phase, e.g. lipids (biota) or organic carbon (sediment). PESs allow reaching equilibrium in biota covering lean and lipid-rich tissues, and thus to compare chemical activity in a set of species across trophic levels. In this study, a small Swedish lake (Ången), which is a well characterized, enclosed ecosystem, has been selected, and nine different species were studied: pike, perch, pikeperch, bream, roach, eel, crayfish, and two species of freshwater mussels. The lipid content of the homogenized samples was measured, an either muscle tissue or the entire body (carcasses) were investigated, from single or pooled individuals, depending on their size and total mass of lipids to ensure non-depletive sampling. In order to calculate their ecosystem-specific trophic level, $\delta^{15}N$ and $\delta^{13}C$ were determined. The homogenized tissues were sampled using silicone sheets with one to three different thicknesses: 125, 250 and 350 µm to confirm equilibrium partitioning between the samples and the silicone, relocating the samplers 6 times per day over 7 days. The pollutants from the silicone sheets were then extracted and submitted to cleaned-up prior chemical analysis. Five sediment samples from the study area were sampled using silicone-coated glass jars, a well-established PES in this matrix. The same approach was tested for water, using a novel device to continuously pump water through the silicone-coated jars. The analysis and quantification of a wide range of compounds, including PAHs, PCBs, PBDEs, pesticides, musk compounds, sunscreens and antioxidants, were achieved with gas chromatography/high-resolution mass spectrometry (GC-HRMS Orbitrap Q-Exactive, Thermo Fisher). The present work accomplishes the challenge of achieving multimedia equilibrium partitioning for a broad range of compounds using PESs in homogenized biota with different lipid content, sediments and potentially water. PESs allow to directly compare (1) the thermodynamic status of contaminants in biota from different trophic levels to assess potential biomagnification and calculate Trophic Magnification Factors (TMFs) and (2) across multimedia biotic and abiotic compartments.

04.11.09 Exploring Personal Chemical Exposures of Structural Firefighters Using Silicone Dog-Tags As Passive Samplers

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Structural firefighters are exposed to many potentially hazardous chemicals while on the job. In a burning building, chemicals in furniture, building materials, and home goods can be volatilized in the high heat, and pyrogenic chemicals can be formed. Adverse health outcomes observed in firefighters, such as increased incidence of various cancers and cardiovascular disease, raise concerns about these occupational exposures. This study uses silicone dog-tags worn around the neck as passive samplers to compare chemical exposures of firefighters when on- and off-duty. There were 57 firefighter participants selected from two stations in the Kansas City Metropolitan area; on average, one 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 concentrations of the chemicals under investigation would be higher in paired on-duty versus off-duty tags, and that the concentrations in high versus low call volume station tags would be higher. It was also hypothesized that dog-tag concentrations would be influenced be firefighter rank, as well as number of fire attacks an individual participated in. Each firefighter wore separate dog-tags while on- and off-duty for a total of 30 24-hour shifts. Firefighters also filled out questionnaires, indicating the number of fire attacks they participated in, rank in their department, plus other demographic and lifestyle information. The dog-tags were extracted and analyzed using GC-MS for polychlorinated biphenyls (PCBs) and volatile organic chemicals (VOCs). Preliminary data shows that 16 different firefighters had detectable concentrations of PCBs in at least one of their dog-tags. 12 different PCB congeners were detected, with PCB 153 being the most frequently detected. Additionally, 40% of high call volume station firefighters and 15% of low call volume station firefighters had detectable PCB exposures, supporting the hypothesis that the high call volume station firefighters had greater exposures. Patterns between paired on- and off-duty tags appear to be more complex. 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.

04.11.10 Assessing Chemical Movement and Temporality at a Former Creosote Site

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Bioavailable chemicals are moving constantly in the environment. Chemical movement will affect the assessment of exposures, remediation and interventions. In this study, we present the development of a field sampling approach to measure diffusive and advective chemical movement across a contaminated site at multiple time points. Diffusive flux describes the movement of chemicals from a compartment with a higher concentration to a compartment with a lower concentration. Advective flux describes the bulk movement of water and its transport of contaminants from one environmental compartment to another. Temporal impacts, such as seasons, likely also play a role in the direction and magnitude of chemical movement. We co-deployed passive sampling devices (PSDs), measuring advective and diffusive flux, with seepage meters at eleven locations at a former creosote site. PSDs were located at the sediment–water and water–air interface. Sampling took place in August, September and October of 2019 and November and December of 2020.

Samples were extracted analyzed by gas chromatography triple-quadrupole mass spectrometry for 65 individual parent and alkylated polycyclic aromatic hydrocarbons (PAHs) and alkylated PAHs used for forensic source determination. The largest contributors to chemical movement across site and season were two, three and four ring PAHs and alkylated naphthalene PAHs. Diffusive and advective flux changed in both direction and magnitude temporally by up to seven orders of magnitude. Deposition from air to water was larger than volatilization from the water, suggesting new atmospheric inputs into the system. Diffusive flux magnitude was, on average, three times larger than advective flux for all locations. Chemical movement of PAHs was dominated by water-porewater diffusive flux for all sampling time points.

04.12 Where the Tire Rubber Meets the Road: Detection, Toxicity and Management of Tire-Wear Microplastic

04.12.01 How Concerned Should We Be About Tire Particles? - Fluxes from Roads to the Marine Environment

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Tire wear particles are generated due to friction between the tire and the road surface and are assumed to present a major source of microplastic emissions to terrestrial and aquatic environments. It has been estimated that tire wear could account for 65% (18,000 tones annually) of all microplastics released to UK surface waters. Despite their apparent significance, data on the pathways for tire wear particles from roads to the marine environment is sparse. On a global scale, only around 1% of studies report finding any tire particles at all. This contradiction is concerning because tires contain a range of potentially hazardous chemicals which have been shown to cause harm to marine life. Here we quantify tire particle fluxes to the marine environments away from roadways. For this, 96 samples have been collected from Plymouth and Bristol, two coastal cities in the UK. Examined particle pathways include: (1) Wastewater effluents, (2) Storm water runoff, and (3) Airborne tyre particles next to roadsides. All samples were analyzed using Pyr-GC-MS to determine tire wear mass using N-cyclohexyl-2-benzothiazolamine as a marker. In addition, a subset of tire particles was isolated and exanimated by visual identification to obtain particle characteristics. Tire wear was present in each of the three pathways examined, detected within 88 % of samples, with concentrations of up to 8.2 mg/L collected from storm water drains. For the Plymouth area, back-of-the-envelope calculations suggest a tire wear flux through storm water runoff of up to ~600 tones annually. In contrast, wastewater effluents showed much lower concentrations of up to 0.3 mg/L. Airborne tire particle concentrations were generally higher in closer proximity to roadways with highest concentrations detected next to motorways with, up to 97 mg tire particles per meter square. Our study suggests that storm water drains present a major pathway for tire wear to the marine environment, while wastewater effluents may be of lesser importance. In addition, airborne particle emission might be of significance in areas where roadways are in close proximity to the coastline or river systems. Future work should examine the fate of tire particles in the marine environment and identify potential accumulation hot spots in seafloor sediments. Knowledge on tire wear fluxes, concentrations, and accumulation zones are essential to determine ecotoxicological risks imposed by tire wear to the marine environment.

04.12.02 Tire and Road Particles - a Novel Method for Quantification Using Pyrolysis Gas Chromatography Mass Spectrometry

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Tire and road wear particles (TRWP) may constitute the largest source of microplastic particles into the environment. Quantification of TRWP emissions are associated with large uncertainties which are in part due to inadequate analytical methods. A new methodology is presented to improve the quantification of TRWP concentrations in environmental samples, using Pyrolysis Gas Chromatography Mass Spectrometry (Pyr-GC-MS). Pyr-GC-MS analysis of Styrene Butadiene Styrene (SBS), a component of PMB used on road asphalt, produces pyrolysis products identical to those of Styrene Butadiene Rubber (SBR) and Butadiene Rubber (BR), which are used in tires. The proposed method uses multiple pyrolysis products (*m/z* 78 Da for *Benzene, m/z* 118 Da for *α-methylstyrene*, *m/z* 117 Da for *M-ethylstyrene* and *m/z* 91 Da for *Butadiene trimer)* to quantify the combined mass of SBR, BR and SBS rubber in a sample. This combined mass is then used in a series of calculation steps in order to calculate the contribution of tire and PMB in the sample. These calculations use traffic data from each sample location, such as the Annual Daily Traffic and specified tire concentrations adjusted to the location and the season. This includes for example adjusting for ratio of personal vehicles to heavy vehicles, or the use of studded winter tires or non-studded winter tires, when calculating the tire contribution in the sample. The calculations can be adjusted to the available traffic information, and together with the use of multiple markers and specified tire concentrations, provides a novel and widely applicable method for quantifying tire and PMB rubber in environmental samples. Using recovery tests, the performance of the method showed recoveries of 83-92% for a simple matrix (tire) and 88-104% for a complex matrix (road sediment). The use of the method was further demonstrated with three different environmental samples; road-side snow, road-side soil and gully-pot sediment. The concentrations of tire ranged from 0.1-17.7 mg/mL (snow) to 0.6 – 68.3 mg/g (soil/sediment) and the concentrations of PMB ranged from 0.03-0.42 mg/mL (snow) to 1.3- 18.1 mg/g (soil/sediment).

04.12.03 Tire and Road Wear Particles in the Environment: A Morphological Signature

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Tire and Road Wear Particles (TRWPs) are emitted from the interaction between roads and tires during their use, resulting in elongated (cigar-shaped) particles with mineral incrustations derived from the road [1]. The quantity of tire wear released every year is estimated to be 6M tons worldwide[2]. Despite this significant amount, TRWPs are today sparsely studied and the research of their impact on the environment is yet to be developed. The aim of this work was to evaluate qualitatively the presence of TRWPs on roads but also in the surrounding environment. Samples were collected directly on roads, in rainwater drainages and in ditches at a further distance from the road, as well as at the border of a field. They were sampled on multiple occasions over a one-year interval. TRWPs were separated from the environmental matrix using densitometry. The isolated TRWPs were analyzed by optical microscopy and scanning electron microscopy (SEM). TRWPs were observed in all of the sample sites. We investigated the number of particles, the particle size and morphology as a function of the distance from the road. Differences and similarities have been observed between the samples.

04.12.04 Evaluation of Stormsewer Inlets to Capture Low- and High-Density Tire Wear Particles From Roadway Runoff

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Tire wear particles (TWPs), emitted due to the friction of tires on road surfaces during vehicle driving and breaking, are increasingly being recognized as a dominant fraction of microplastic in traffic-affected areas. TWPs vary in density, size, shape, and composition. Tire material has long been a subject of ecotoxicity assessment due to the potential for toxic leachates Recent studies have linked TWP leachates from storm events to acute mortality episodes in Coho Salmon. Because of their toxic properties, containment, and prevention of TWP from entering the environment should be a priority. It is known that TWPs are abundant in roadside soils and stormwater runoff and may accumulate in rain gardens and stormwater ponds. However, more research is needed to examine the fate of TWPs in grey-stormwater infrastructure. This study investigated how effective newer stormsewer inlet devices, also called manufactured treatment devices (MTDs), are in capturing TWPs. For this study, sediments were sampled from the chambers of eight MTDs in Mount Pleasant, SC. The 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 sites in ditches and tidal creeks were also taken. Density separations were performed using a supersaturated NaCl solution and sodium polytungstate to distinguish between the low- and high-density fractions. The TWP in these fractions were counted through TWP identification microscopy. The concentrations of TWPs in different size and density classes were compared between the roadway sources, the stormsewer inlet devices, and their environmental outfalls. In a preliminary analysis from two study sites, the range of low-density (< 1.2g/cm^3) tire wear particles were 80-300 particles/g dry wt. For high-density particles (1.2-1.9 g/cm^3) the amount of tire wear particles ranged from 150-400 particles/g dry wt. The amount of low- and high–density particles captured in stormsewer inlet devices are still under evaluation, but early results show that concentrations are ~2x higher in MTD sediments than in road dust. We will compare the capture of TWP by different MTD designs and will also assess concentrations of TWP indicator chemicals such as zinc for this investigation to report on the effectiveness of stormsewer inlet devices to prevent TWPs entry into the environment.

04.12.05 Linking Occurrence of Tyre Road Wear Particles (TRWP) and Tyre Additive Chemicals in Australian Urban Stormwater

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Tyre road wear particles (TRWP) have been suggested as one of the primary, yet vastly understudied, sources of microplastic to the environment. Further concern arises from chemical additives incorporated into the tyre material that can leach into the surrounding environment. These chemicals have demonstrated links to aquatic toxicity including the vulcaniser hexa(methoxymethyl) melamine (HMMM) and the antioxidant derivative 6PPD-Quinone, with particularly high concentrations in stormwater runoff. The current study investigated inputs and co-occurrence of TRWP and a wide range of tyre additive chemicals (including HMMM and 6PPD-quinone) in an Australian urban water cycle during severe storm events in 2020. Grab samples were taken from a creek in Brisbane, QLD, which has direct traffic inputs from two major artery roads through the city. Samples were filtered to remove particles > 0.7 µm and the residue analysed with Pyrolysis gas chromatography mass spectrometry for markers of TRWP, including synthetic and natural rubber. The filtrate was extracted with solid phase extraction and analysed with LC-MSMS for a range of tyre related chemicals (n=16), including the new chemical of concern 6PPD-Quinone. A similar chemical profile was seen throughout every storm event, enabling a fingerprint of Brisbane traffic related chemical inputs to be established. The chemicals of high concern, HMMM and 6PPD-Quinone were detected in all samples, all be it at concentrations lower than those previously reported to have links with aquatic toxicity. Furthermore, the mass of synthetic rubber (as a proxy for TRWP) in the samples correlated with concentrations of the tyre related chemicals, providing the first evidence of a direct link between TRWP inputs and chemical tyre additive profile in an urban water cycle.

04.12.06 Microplastics in Stormwater Ponds and Their Adjacent Receiving Tidal Creeks in Coastal South Carolina: A Focus on Microscopic Tire Wear Particles

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Microplastics (< 5 mm) are ubiquitous in coastal environments. Recent attention has turned to highly abundant microscopic tire wear particles, produced through tire abrasion, in aquatic environments including the Charleston Harbor watershed of SC, USA. Stormwater ponds are a type of green infrastructure that detain stormwater runoff and allow a short retention time for particle settling. They are therefore expected to be able to trap microplastic and tire wear particles; conversely, they may also serve to transmit them via pond discharge points, connecting upland sources to coastal waters. The objective of this study is to determine the contribution of stormwater detention ponds to microplastic and tire wear particle abundance in tidal receiving waters. Sediment and water samples were collected from locations within five stormwater detention ponds and tidal creeks, including within the pond near the inlet and outlet points, in the tidal creek at the pond discharge point, and up to 50 m downstream of the discharge point. Sediment samples underwent a density separation using supersaturated NaCl solution, with smaller subsamples going through a two-stage density separation using supersaturated NaCl solution and sodium polytungstate solution to extract higher-density tire wear particles. Sediment and water samples were digested, then analyzed for microplastic abundance and composition under a dissecting microscope. We intend the results of this research to be used to inform coastal decision makers on the potential for pond infrastructure and management to mitigate downstream microplastic transport and impacts.

04.12.07 Deposition of Tire and Road Wear Particles in Urban and Highway Snow Banks

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Snow along urban road areas are known to accumulate contaminants, especially metals such as zinc (Zn), lead (Pb) and particles. Recent studies suggest that snow banks also acts as a medium that traps microplastics, both in urban areas as well in remote areas. Tire and polymer-modified bitumen particles both contain synthetic rubbers (Styrene Butadiene

Rubber+Butadiene Rubber in tires; Styrene Buadiene Styrene in PMB), defining them as microplastic particles. In this study, recently developed Pyr-GC-MS method for quantifying these rubbers was applied (submitted as a poster abstract: "Tire and road particles – a novel method for quantification using Pyrolysis Gas Chromatography Mass Spectrometry"). Snow samples were collected February 2019 from 11 sites in total, including the inner (Ring road 2) and outer (Ring road 3) city of Oslo in Norway, and major road site locations outside of the city centre. The sample sites represent different areas of the city and different traffic characteristics (e.g. traffic density and speed limits). Samples were taken as snow cores at 0, 1 and 3 meters from the road in order to study the dispersion from the road to the sides. The study aims to give valuable information on the factors responsible for variations in tire and PMB concentrations at different sites using snow as the sample matrix, and provide new data on the levels of tire and PMB particles in meltwater, assessing this source into the environment with other road-related sources from previous studies. To obtain these aims, the results were used in two approaches: 1) calculated as mass load (mg/m^2) to investigate the variation of total rubbers from tire and PMB and an in-depth analysis of the possible explanatory factors contributing to this variation, such as traffic density, speed or type of road, and 2) calculated as melted snow (mg/L) to evaluate the contribution of tire and PMB particles from snow to the environment and compare this source to other input sources. The preliminary results show that the concentrations of rubbers in mass load vary between 63 and 13 831 mg/m^2, with the Average Annual Daily Traffic for heavy vehicles and the speed limit as the most significant explanatory factors for the observed variation. For concentrations in melted snow, tire particles ranged from 75 to 15664 mg/L and PMB particles from 23 to 4860 mg/L, with a significant decrease in tire concentrations from 0m to 3m distance from the road (Redundancy Analysis R2=0.18, p=0.016).

04.12.08 Rising Seas and Roadway Debris: Environmental Fate of Tire-Wear Microplastic Following Nuisance Flooding in Coastal South Carolina

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The low-lying streets of Charleston, SC experience nuisance flooding more than 50 days annually due to encroaching high tides. This frequent tidal flooding may transport microplastic particles (MP), including tire wear particles (TWP), away from the road surface into adjacent tidal creeks or marsh sediment. The objective of this research was to establish baseline data on the abundance of MP and TWP in street floodwater and to elucidate the environmental fate of TWP following nuisance flooding. Street locations around the Charleston peninsula were sampled during flood events, and their adjacent tidal creeks were opportunistically sampled both before and after predicted flood events. Floodwater contained 379 ± 99 (avg ± SE) MP/L, which is significantly higher than the average of 6.6 MP/L found in Charleston Harbor. Most MP (82.4%) in floodwater were suspected TWP. However, adjacent tidal creek water did not contain more TWP after flooding compared to before. This suggests that TWP in street floodwater may not be immediately transported into adjacent tidal creeks, but rather deposited in nearby marsh sediment. The environmental fate of TWP is determined by particle properties such as size, density, and mineral encrustations from the road surface. To better understand the range of these particles' densities, TWP (< 500μm) were collected from the street surface and placed in solutions of various densities (0.8-2.9 g/cc). Floating particles were analyzed using SEM-EDX microscopy to identify encrusted minerals, which affect particle density and therefore determine how these particles are transported by ebbing floodwater and marsh sediment. Additionally, marsh sediment was analyzed for TWP. Preliminary results suggest that TWP abundance is substantially higher in street-side marsh sediment (18,088 TWP/kg) than in creek-side sediment (1,152 TWP/kg). Understanding the pathways by which street-associated TWP are transported to coastal waters by nuisance floodwater and the role that marsh sediment may play in capturing microscopic roadway debris is critical to developing science-based management decisions to mitigate future impacts of sea level rise.

04.12.09 Occurrence and Temporal Trend of the Coho Salmon Toxicant in Urban Creeks

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Coho salmon in the Pacific Northwest suffer from acute mortality after exposed to urban stormwater. Our recent work has identified the coho salmon toxicant (6PPD-quinone) as a transformation product of a ubiquitous tire rubber additive (6PPD). 6PPD-quinone is acutely toxic to juvenile coho salmon, and it has been found in all the highway runoff samples and some urban creek stormwater samples. Based on our previous semi-quantification method of 6PPD-quione, we developed a more accurate quantification method using HPLC-MS/MS (triple quadrupole) and an isotope-labeled internal standard, and tested its performance on environmental samples. To better understand its environmental occurrence and temporal trend during storm events, we collected stormwater samples from three urban creeks, across seven rain events. An ISCO sampler was applied to automatically collect high-resolution temporal profile samples (one sample per hour) in Miller Creek. Grab samples were collected from Longfellow Creek and Thornton Creek to confirm the widespread occurrence of the 6PPD-quinone. Hydrographs and pollutographs of 6PPD-quinone were paired to analyze the factors influencing its environmental transport. This work helps us to understand the environmental behavior of the coho salmon toxicant, and provides insights for stormwater treatment and salmon conservation.

04.12.10 A Direct Mass Spectrometry Method for the Rapid Analysis of a Ubiquitous Tire Derived Toxin N-(1,3-dimethylbutyl)-n'-Phenyl-P-Phenylenediamine Quinone 6-PPDQ

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A recently discovered ozonation product of a tire preservative, N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine quinone (6-PPDQ), threatens Coho salmon populations in the pacific northwest at ng/L concentrations. Sensitive and selective analytical techniques are needed to study the environmental fate and distribution of 6-PPDQ including identification of multiple sources and potential mitigation strategies. Conventional analysis such as those based on liquid chromatography-mass spectrometry (LC-MS) require sample clean-up, which can increase the analytical duty cycle and the cost of consumables. Given the expected widespread occurrence of this toxin in stormwater run-off, high throughput analytical methods are desirable. Condensed phase-membrane introduction mass spectrometry (CP-MIMS) provides a rapid and convenient online separation of hydrophobic analytes from ionic and particulate components in complex samples using a semi-permeable polydimethylsiloxane (PDMS) membrane. Permeating analytes dissolve in a flowing solvent and are directly infused into a mass spectrometer in real-time. We report quantitation of 6-PPDQ using positive electrospray ionization (ESI) coupled to tandem mass spectrometry with detection limits below 50 ng/L using a low internal volume, capillary hollow fiber membrane directly immersed in the sample. Formic acid added directly to the sample improves perm-selectivity for 6-PPDQ (membrane rejects amines with $pK_a > 4$) and enhances the ionization efficiency at the ESI. The complete analytical duty cycle is currently < 15 mins and can be shortened using thinner membranes. High resolution, accurate mass (Orbitrap Exploris 120) confirmed the identity of the permeating product and fragment ions. Quantitative work employed a triple-quadrupole MS (QSight 220), where ion ratios for several 6-PPDQ fragments were used as qualifier ions and tandem MS (299 □215) is employed for quantitation. We report the analysis of 6-PPDQ in a variety of constructed and real-world samples including stormwater, surface water, and seawater by CP-MIMS. Further, we employ this technique as an in-situ process monitoring strategy to directly observe the dynamic changes in the aqueous phase concentration of 6-PPD and 6-PPDQ in complex, heterogeneous samples. Overall, direct membrane sampling provides a fast, convenient MS strategy with little compromise to selectivity or sensitivity and the ability for high throughput and real-time reaction monitoring.

04.12.11 Physicochemical Property, Transport, and Transformation Estimation of 6ppd-Quinone from Molecular Structure

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6PPD-quinone, a transformation product of a globally ubiquitous tire rubber antioxidant, was recently discovered to be responsible for decades of acute mortality events in coho salmon (Oncorhynchus kisutch) in the U.S. Pacific Northwest. Coho salmon returning to spawn in freshwaters are exposed to this contaminant via stormwater influx, resulting in rapid mortality of 40-90% of returning salmon in urbanized watersheds with extensive impervious surfaces. Analysis of stormwater runoff and receiving waters across the U.S. West Coast indicates that concentrations in the environment (< 3 to 19 µg/L) often exceed acute mortality thresholds for coho salmon (0.8 µg/L)1. Due to the recency of the discovery, the environmental fate and toxicity of 6PPD-quinone in aquatic ecosystems remains unknown. In this study, the simplified molecular-input line-entry system (SMILES), universal quasichemical functional group activity coefficients (UNIFAC), the UFZ-linear solvation energy relationship (LSER), and enviPath were used to predict the physicochemical properties and transformation products of 6PPD-quinone based on molecular structure. These predicted properties were used as default parameters for estimating partitioning between different environmental compartments in a level III fugacity model. Parameters including water depth, fraction of organic carbon, and water temperature were varied for sensitivity analysis. The results of this study provide a big picture overview of the potential fate of 6PPD-quinone in aquatic ecosystems.

04.12.12 Toxicity of Novel Tire-Derived Chemical 6ppd-Quinone to Coho Salmon (Oncorhynchus kisutch) Under Varying Environmental Conditions

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Coho salmon (*Oncorhynchus kisutch*) returning to spawn in urban-impacted watersheds of the Pacific Northwest of North America suffer high rates of mortality from exposure to stormwater runoff. Juvenile coho salmon can serve as a surrogate for studying the observed mortality on adult spawners. Using juvenile coho, we recently identified a novel tire-derived chemical (6PPD-quinone) as the primary contaminant in roadway runoff responsible the acute mortality. Acute lethality was determined to be on the order of < 1 µg/L under standard laboratory rearing conditions for juvenile coho salmon. Previous exposures were conducted under static 24-h conditions. Conditions in streams often differ from those in the laboratory, including differences in flow, temperature, and water chemistry. Many of these differences can affect the bioavailability and/or toxicity of chemical contaminants. To extrapolate lab-derived toxicity data for 6PPD-quinone to expected toxicity in the field, we will determine toxicity to juvenile coho under varying water quality (e.g., temperature, pH, ionic strength, dissolved organic matter) and flow conditions. This data will help elucidate what receiving water conditions result in maximal concentrations of bioavailable toxicant and put further at risk coho salmon and other vulnerable aquatic organisms.

04.12.13 The Internalization and Sub-Lethal Effects of Tire Wear Micro and Nanoparticles on Two Estuarine Indicator Species

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Plastic debris is a ubiquitous source of pollution in estuarine ecosystems. Micro (< 5 mm) and nanoplastics (< 1µm) are known to have adverse effects on the habitats, diets, and physiologies of aquatic organisms, but questions remain about the relative risk across salinities, concentrations, and sizes. Less is known about the effects of tire wear particles (TWP), recently defined as plastic debris, as an aquatic contaminant. TWP is generated from automobile traffic, composed of complex mixtures of oil, plastic, steel, and additives and has been detected in coastal and estuarine habitats. Herein, early life stages of estuarine indicator species inland silverside (*Menidia beryllina*) and mysid shrimp (*Americamysis bahia*) (1 day pre-hatch – 4 days post hatch; 7-14 days post hatch, respectively) were exposed to three concentrations of TWP (60, 6000, and 60,000 particles/mL) at micro and nano size fractions with leachate across 5-25 ppt salinity gradient. *M. beryllina* and *A. bahia* had significantly altered swimming behaviors, such as freezing, in zone duration and total distance moved, that could lead to an increased risk of predation and foraging challenges in the wild. Both *A. bahia* and *M. beryllina* growth was reduced in a concentration dependent manner when exposed to micro TWP, whereas *M. beryllina* demonstrated reduced growth only when exposed to nano TWP. Salinity related TWP internalization were also observed with both taxa. *A. bahia* and *M. beryllina* had significantly higher particle ingestion at 15 PSU following micro TWP exposure. The presence of adverse effects in *M. beryllina* (growth at micro TWP and behavior) and *A. bahia* (behavior) *indicate* that even at current environmental levels, which are expected to continue to increase, aquatic ecosystems are likely experiencing negative impacts from TWP pollution.

04.12.14 Micro and Nano Tire Wear Particles Cause Mortality and Abnormalities in Model Organisms Danio rerio and Daphnia magna

B. Cunningham, B.J. Harper, S.M. Brander, S.L. Harper, Oregon State University / Environmental and Molecular Toxicology

Annual production of plastics has grown to several hundred million tons, nearly 80% of which will eventually find its way into the environment, where it may be internalized by aquatic organisms. Environmental sampling has found a diversity of plastic types including high levels of black rubber— generally identified as tire debris. Tire wear particles (TWPs) originate from friction of tires on roads and are composed of a variety of materials, each carrying their own potential toxicity. On average, it is estimated that the US generates 1,524,740 t/yr of TWPs. The magnitude of the impact of this flow of TWPs to the environment is not yet known. Available literature focuses on the toxicity of leachate, overlooking potential effects of the TWPs themselves. We conducted experiments to assess particle and/or chemical toxicity of micro (1-20µm) and nano (< 1µm) TWPs to two model organisms, zebrafish *Danio rerio* and crustaceans *Daphnia magna*. To assess effects on development, zebrafish embryos were exposed to concentrations of TWPs or leachate ranging from 1.0 x 10^3 to 3.0 x 10^9 particles/ml (n=18, four replicates). Exposures began 8 hours post fertilization (hpf) and lasted a total of 5 days, with assessments at 24 and 120 hpf. In embryonic zebrafish, high concentrations of all tire exposures significantly decreased spontaneous movement at 24 hpf. Greater mortality and sublethal malformations were observed following nano TWP and leachate exposure as compared to micro TWPs. Unique abnormalities in the nano exposures indicates that there is particle-specific toxicity. This is some of the first research to show that exposure of zebrafish to nano TWPs, has detrimental effects on survival and development. The survival results were different for a 48 hour exposure of neonate *D. magna* to TWPs or leachate, ranging from 1.3 x 10^5 to 3.3 x 10^9 particles/ml (n=15, three replicates). LC_{50} calculated for micro and nano TWPs were 4.7 x 10^5 and 3.0 x 10^8, respectively. Much of the particle mortality could be explained by toxicity elicited by the leachate, as no particle-specific effects were noted for *Daphnia*. Overall, these two aquatic species appear differentially susceptible to TWPs entering the environment. It is important to identify the differing toxicities of TWPs for different species to achieve a full understanding of the impacts they may have on an ecological community as a whole.

04.12.15 Toxicity of a Pulsed Chronic Exposure to Crumb Rubber Particles in Mummichog (Fundulus heteroclitus)

S.B. LaPlaca, P. van den Hurk, Clemson University / Biological Sciences

Microrubber (MR) encompasses all tire-related particles in the microscale and has recently been acknowledged as a microplastic class. While tire particles have been entering the environment since the introduction of rubber tires for vehicles, the concern regarding tire wear particles (TWP) as an environmental contaminant is relatively new. Recent studies have examined physical and chemical toxicity of MR and leachates to a variety of organisms. However, there is a lack of information on the long-term effects of tire particle exposure under environmentally realistic conditions. The current study examines the chronic toxicity of crumb rubber (CR) particles to the estuarine fish species, mummichog (Fundulus heteroclitus), under a pulsed-dosing regime at environmentally relevant concentrations. Organisms were individually exposed to CR (38 – 355 µm in size) at concentrations up to 0.25 g/L every five days for 42 days total. Previous research has demonstrated that adult mummichogs can survive at concentrations up to 6.0 g/L CR. Therefore, the focus of the present study was on sublethal effects. Bile fluorescence was measured as an indicator or exposure to polycyclic aromatic hydrocarbons (PAHs) from CR. Oxidative stress was measured through the TBARS assay, free glutathione (GSH), and oxidized glutathione (GSSG). DNA damage was measured through the formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG). Initial results indicate an increase in bile fluorescence as CR concentration increases, suggesting an increase in exposure to PAHs from CR which agrees with previous findings from our lab. Preliminary data for DNA damage shows greater DNA damage at higher CR concentrations. We hypothesize that sublethal effects such as increases in oxidative stress biomarkers and DNA damage result from long-term exposure to CR, even at low concentrations. The results from biomarker analyses will be discussed as well as overall conclusions on chronic effects of CR exposure to mummichogs.

04.12.16 Research Priorities Around Tire-Related Water Pollution

K. Moran, San Francisco Estuary Institute; A. Gilbreath, San Francisco Estuary Institute / Watershed Program; E. Miller, M. Mendez, D. Lin, R. Sutton, San Francisco Estuary Institute

Every vehicle on the road sheds tiny particles from its rubber tires into the environment. As they disperse into the environment, these microplastic particles convey tire tread ingredients into the air, into runoff, and eventually into surface waters. Recent investigations in the San Francisco Bay Area identified urban runoff as a major pathway for microplastics to enter receiving waters, with black rubbery particles from tires estimated to be the single most common microplastic flowing into the Bay. Modeling studies indicate tire wear may be one of the top sources of microplastics to the environment globally. Tire particles and associated contaminants pose toxicological risk to aquatic species. Chemicals that leach from tire tread also appear in urban runoff. One of these chemicals, 6PPD-quinone (a degradate of a tire antioxidant) causes pre-spawn mortality in coho salmon. Based on conceptual models to synthesize and integrate our current understanding of tire particle sources and pathways to urban runoff, we identified several key data gaps crucial to identifying and designing tire wear pollution prevention and mitigation actions. For example, particle surface area measurements would determine the particle sizes that have the greatest potential to release potentially toxic tire-related chemicals into urban runoff and surface waters. Such information has potential to modify the design and location of mitigation strategies. We also identified tire particle characterization and tire formulation information with broad implications for environmental monitoring and toxicity study designs.

04.12.17 Prevention and Mitigation of Tire-Related Pollution in Urban Runoff

K. Moran, San Francisco Estuary Institute; A. Gilbreath, San Francisco Estuary Institute / Watershed Program; E. Miller, M. Mendez, D. Lin, R. Sutton, San Francisco Estuary Institute

Recent investigations in the San Francisco Bay Area identified urban runoff as a major pathway for microplastics to enter receiving waters, with black rubbery particles from tires estimated to be the single most common microplastic flowing into the Bay annually. Tire particles and associated contaminants pose toxicological risk to aquatic species. Based on conceptual models to synthesize and integrate our current understanding of tire particle sources and pathways to urban runoff, we developed a broad framework of control strategies for tire particles and the water pollutants that they carry. The goal of the control strategy framework is to inform future research and management recommendations for managing tire-related water pollution. There are many available approaches to prevent and mitigate tire-related water pollution, from re-formulating tires to remove toxic ingredients, to reducing tire wear rates, to collecting wear debris at the point of emission from vehicles, to downstream collection and treatment of urban runoff to remove tire particles and chemicals. These control strategies highlight the wide range of opportunities to prevent and mitigate tire-related water pollution, and the necessary roles for the vehicle industry and its suppliers, government, and consumers.

04.12.18 Regulating Chemicals of Concern in Tire Rubber - a California Approach

A.C. Doherty, California Environmental Protection Agency / Department of Toxic Substances Control

There is growing concern about the presence of microplastics, including microrubber, in the aquatic environment due to the potential for physical and chemical impacts to aquatic organisms. Recent research has identified an ever-growing number of tire-derived chemicals that may be of concern. The continuous release of tire wear particles to the aquatic environment ensures a constant source of these chemicals and an increased potential for exposure to aquatic organisms. California Department of Toxic Substances Control's Safer Consumer Products (SCP) program has begun to evaluate and propose regulations to mitigate the effect of the presence of some of these chemicals, including zinc and 6PPD. SCP's 2021-2023 Priority Product Work Plan added motor vehicle tires as a new category to be evaluated under the SCP framework and expanded the scope of a category carried over from the prior Priority Product Work Plan — Building Products and Materials Used in Construction and Renovation — which will allow for evaluation of synthetic turf. SCP's precautionary approach allows the program to move relatively quickly to require manufacturers to evaluate safer alternatives to chemicals of concern, as evidenced by the proposal to list motor vehicle tires containing 6PPD as a Priority Product. SCP also has a menu of regulatory responses it can impose to mitigate the adverse impacts of exposure to chemicals in the consumer products it regulates. However, important data gaps need to be addressed before the program can regulate many chemicals whose presence in stormwater has been attributed to tires, including benzothiazoles, chlorinated paraffins, octylphenol and octylphenol ethoxylates, 1,3-diphenylguanidine, and PAHs. These include the chemicals' presence and function in tires, their leachability from tires under environmental conditions, the relative importance of tires as a source to the aquatic environment, environmental monitoring data, and toxicity. This talk will provide a brief overview of the SCP program and the information required for SCP to regulate chemicals in consumer products. It will also highlight some of the current data gaps around other chemicals of concern in tires. Filling these data gaps is the first step to enable SCP to continue to address the impacts of tire-derived chemicals in aquatic environments.

04.12.20 High Resolution Mass Spectrometry Analysis of Tire Antidegradant Breakdown Product 6ppd-Quinone in Coho Salmon (Oncorhynchus kisutch), Aqueous and Solid Matrices

A.N. Patterson, R. Hrabak, Eurofins Environment Testing America

The recent publication by the University of Washington (UW) in the scientific journal *Science* was a breakthrough in understanding one of the challenges wild-run salmon face. Every spring and fall, millions of salmon up and down the West Coast voyage from the Pacific Ocean back into their native rivers and streams to spawn. Unfortunately, significant numbers of Coho salmon never make it to the spawning grounds and instead perish for no apparent reason. The breakthrough at UW was identifying the acutely toxic compound to the salmon: 6PPD-quinone. The parent compound, 6PPD (*N*-(1,3-dimethylbutyl)-*N'*-phenyl-1,4-benzenediamine) is a tire additive designed to prevent tires from breaking down. In the very process of protecting tires from oxidation, 6PPD is transformed into 6PPD-quinone, which is unknowingly toxic to Coho salmon. As tires wear, the worn rubber is continuously deposited on roadways. This deposited material invariably makes its way into waterways through runoff from rains. Although rain naturally dilutes the runoff, the 6PPD-quinone is found to be toxic at the low concentrations found within streams and rivers. It is still unknown how widespread this compound is in the environment or the toxicological impact to other salmonids. This study aims to characterize sediments and aqueous samples for 6PPD-quinone from rivers and streams in Northern California. Attempts will be made to collect and analyze stormwater if rain events occur. The minimum reporting limit of aqueous samples will be 10 ppt and sub-ppb for sediments. Analysis will take place on an LC-QToF-MS utilizing multiple reaction monitoring (MRM) in high resolution acquisition (MRM-HR) and will employ isotope dilution quantitation. Detection of 6PPD-quinone in juvenile Coho salmon (Oncorhynchus kisutch) will also be explored. These samples originated from ongoing toxicological studies under controlled laboratory conditions where they were exposed to known concentrations of 6PPD-quinone. Extraction of various tissues within the Coho will be attempted using sonication and SPE techniques utilizing isotope dilution quantitation. Expected detection limits should achieve 0.25ppb for 6PPD-quinone. Compound screening for metabolites of 6PPD-quinone will be attempted using data-independent acquisition that will provide both full MS and MS/MS fragmentation for all detectable features.

04.13 Chemistry and Exposure Assessment

04.13.01 A 3d-Hydrodynamic Ocean Simulation at Waters Off Niihama in Seto Inland Sea

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As the first step toward a final goal for assessing the safety of chemicals to aquatic organisms, a high-resolution hydrodynamic ocean model was applied to waters off Niihama in the middle of the Seto Inland Sea (SIS), Japan. Into this model, the effects of Class B river and spatial distribution of water transparency were incorporated. Using this model, tidal current, water temperature and salinity were calculated in the area covering the middle of SIS, and then the detailed behaviors at the waters off Niihama were simulated. The simulation was performed in summer when a convective mixing was suppressed and the effect of released chemicals to aquatic organisms was highly concerned. The model well reproduced spatial and temporal variations of tidal current, water temperature and salinity observed at the waters off Niihama. The results from simulation demonstrated that the water exchange by residual tidal currents in the waters off Niihama was faster and the vertical water displacement by tidal currents at steep bathymetry was larger in comparison with in the middle of SIS.

04.13.02 Analysis of Hydroxylated Polycyclic Aromatic Hydrocarbon Metabolites (OHPAH) in Bile of Fish Exposed to PAHs

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Polycyclic aromatic hydrocarbons (PAHs) are formed by two or more fused aromatic rings with or without carbon-substituted groups connected to these rings (alkylated- or non-alkylated-PAHs, respectively). PAHs are known to be toxic and carcinogenic to aquatic life and are ubiquitously present and widely monitored in biota from urbanized areas or areas of petroleum activities. Differences in the distribution of PAHs measured in biota help us assess the sources and bioavailability of these chemicals. However, fish and other vertebrates can quickly metabolize PAHs into hydroxylated forms (OHPAHs) that, in some cases, are more toxic than the unmetabolized PAHs. Therefore, the analysis of OHPAHs in fish bile helps minimize the risk of underestimating the exposure to these chemicals. While the metabolism pathway of PAHs is fairly well understood, there is a substantial lack in the literature on environmental levels of OHPAHs in fish, which are usually limited to a few metabolites from a reduced group of PAHs. Using liquid chromatography-tandem mass spectrometry (LC-MS/MS), we developed a rapid and accurate method to simultaneously measure more than 35 individual OHPAHs, in addition to several petroleum-related alkylated-OHPAH metabolite homologues. Using this method, we have analyzed English sole (*Parophrys vetulus*) collected in the last 20 years throughout Puget Sound, WA, to assess the recent trends of environmentally relevant and chronic levels of PAHs at urbanized sites. Pacific herring (*Clupea pallasii*) bile samples have also been analyzed and differences in the OHPAH profiling could be observed between herring and sole, potentially due to the diverse PAH availability in the benthic *versus* pelagic zones. In another application, the OHPAH profiling differ in fish exposed to different petroleum products (crude oil *versus* diesel), particularly with the use of alkyl-OHPAHs, illustrating the strength of this method. Our method of analysis for OHPAH has been improving the assessment of PAH exposure in the marine environment as well as helping to understand the toxic mechanisms of PAHs in fish. To the best of our knowledge, these are the first studies where alkylated-OHPAH metabolites were measured in bile of field caught fish samples, which substantially improve the PAH exposure investigations.

04.13.03 Analysis of Polycyclic Musks and Common Fragrance Chemicals in Personal and Environmental Samples Using GC-MS

R. Scott, Oregon State University / EMT; C.C. Ghetu, K.A. Anderson, Oregon State University / Environmental and Molecular Toxicology

Appreciation of pleasant smelling odors and aversion to foul smelling odors has been around since the third millenium. As technology and chemistry developed the mass production of perfume began with nitrobenzene, and in 2020, the perfume market was estimated at 11.8 – 31.4 billion dollars in The United States alone. Currently, there are approximately 4,000 chemicals used to scent products. Of these 4,000 chemicals, 1,200 have been flagged as potential or known "chemicals of concern" in the EU and various other international warning lists. Many fragrance formulations are unknown due to proprietary protections. The unknown nature of these mixtures illustrates the need for the continued development of analytical methodologies for the identification and quantitation of these chemicals. In this study, we developed a method to identify and quantify both modern and legacy fragrances using gas chromatography mass spectrometry (GC-MS). The method was optimized using an Agilent 8890 GC with an Agilent 5977B mass spectrometer, and contains 41 compounds. The calibration range has an average R2 of 0.98 and spans four orders of magnitude, which mimics the range in concentrations seen in environmental samples. This method was validated with laboratory control matrix spikes using silicone wristbands (WB) and low density polyethylene (LDPE) passive samplers. To assess inter and intraday precision and accuracy, both matrices were spiked and analyzed in replicates of at least four across three different days. A storage stability study was also performed using standards stored at 4°C for seven months. To demonstrate the real world application of this method, LDPE samples deployed for 30 days from Portland Harbor Superfund Site and wristbands worn by participants in Eugene, OR and New York, NY were analyzed.

04.13.04 Assessment and Comparison of Total and Bioaccessible Atmospheric Metal in Census Tracts With Different Vulnerability Indices Using Orthotrichum Lyellii As a Bioindicator

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Orthotrichum lyelli is a native moss species common in western North America. Avascular plants such as moss absorb nutrients from the surrounding atmosphere. This allows them to serve as bioindicators of air pollution without the cost or effort associated with traditional deployment and passive sampling methods. Four census tracts in a northern Washington State County were identified to represent a range of vulnerability based on the Washington Tracking Network's social vulnerability index. Twenty moss samples (per tract) are collected every 60 days. There is a range of land uses covered by these tracts including residential, industrial, and retail sites. In addition to investigating the distribution of atmospheric metals from an environmental justice perspective, we are also determining what fraction of atmospheric pollution is likely to be bioaccessible to human lungs. Once sampled, the *O. lyelli* is homogenized to remove impurities and separated into two aliquots for total metal and bioaccessible metal analysis using ICP-MS. Bioaccessibility is determined by exposing the moss for 24 hours at 37°C to Gamble's solution, which is a laboratory simulated lung solution that mimics the ionic strength of human interstitial lung fluid. Sampling for this project began in November 2020 and is expected to conclude November 2021.

04.13.05 Assessment of Ethoxylated Surfactants in Wastewater Effluent, Stormwater Runoff, and Ambient Water of San Francisco Bay, CA

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Ethoxylated surfactants are a broad class of ubiquitous organic environmental contaminants used in a variety of commercial and industrial applications. These compounds have received continued attention over the past several decades, particularly as manufacturing rates increase worldwide and certain sub-classes such as alkylphenol ethoxylate surfactants and their metabolites show acute and chronic toxicity concerns, including estrogenic effects. Occurrence of these compounds in surface water has typically been attributed to discharge of treated or untreated wastewater; however, other potential pathways such as stormwater runoff have not been comprehensively evaluated. This evaluation is especially important for large urban waterbodies that receive multiple inputs. We have quantified several important classes of alcohol and alkylphenol ethoxylated surfactants in San Francisco Bay area stormwater runoff, wastewater effluent, and ambient Bay water to determine concentrations and primary pathways of contamination. We employed high performance liquid chromatography coupled with high resolution mass spectrometry to perform ethoxymer-specific quantitation of long-chain polyethoxylated surfactants based on C12-C16 alcohols as well as octylphenol and nonylphenol hydrophobes. Total ethoxylated surfactant concentrations were detected up to 45 µg/L in treated wastewater effluent, with the majority of sites between 0.003 and 4.8 µg/L for the various ethoxylated surfactant series. Generally, similar sum ethoxylated surfactant concentrations were observed in stormwater runoff; stormwater contamination was more ubiquitous than in wastewater, with nearly all target sites having detected total ethoxylated surfactant concentrations ranging from 0.004 to 4.7 µg/L. Analysis revealed that polyethoxylated surfactant levels in ambient Bay waters, which were relatively low compared to wastewater effluent and stormwater runoff concentrations and limited to only two Bay sites, were likely the result of both stormwater runoff and wastewater effluent inputs to San Francisco Bay. These results highlight the importance of considering multiple routes of contamination for ubiquitous surfactant-derived pollutants in ambient waters.

04.13.06 Assessment of Polyquaternium-10 Polymer Adsorption Isotherms by Activated Sludge Solids

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Polyquaternium-10 (PQ-10) is a polymeric quaternary ammonium salt commonly used as an active ingredient in cosmetic and cleaning products and a chemical present down-the-drain. Because of uncertainties about relevance of partitioning to humic acid, this study was undertaken to better understand the fate of polyquaternary polymers through wastewater treatment plants (WWTP). Outcomes from this study would inform the adsorption of polymeric quaternary ammonium salts compounds to WWTP biosolids. Relatively low and high molecular mass and diverse charge density of PQ-10 materials were tested individually to determine if sorption characteristics were mass and/or cationicity dependent. The relationships between dosed amounts and equilibrium concentrations in the aqueous phase (dose-response curves) for each tested polymer indicated that solution concentrations at relatively low dosing (10 - 30 µg (active ingredient (a.i.)/mL) were negligible ("total adsorption" region of dose-response curve), within the limitation of the analytical method used to determine aqueous phase concentrations. Light absorbance of the samples prepared for determination of polymer concentration using a phenol method within this region was close or below to the absorbance of the blank (0 µg (a.i.)/mL), resulting in calculated concentrations near zero. At some level of dosing, subsequent increase in polymer dosage resulted in a quantifiable increase of solution concentration ("solution concentration rising" region). The relationships between concentration of the polymers in the aqueous phase and concentration of adsorbed polymer (adsorption isotherms) were non-linear. The ability of activated sludge solids to bind tested polymers may be characterized by apparent "threshold values" of the loading for each polymer. Below the "threshold value" solution concentration of the polymer is close to zero and above this value it becomes measurable by a phenol spectrophotometric method. Results from this and related studies, strengthens evidence of significant sorption and removal of these materials in WWTP.

04.13.07 Commercial Fish Feed for Toxicological Studies Contains Background Levels of Per- and Polyfluoroalkyl Substances (PFAS)

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Toxicological studies commonly involve dietary exposures in which test chemicals are added to animal diets (e.g., feed) to achieve target dietary exposure concentrations. Zebrafish (Danio rerio) is the very popular model for dietary exposure studies. To our best knowledge, there are no reports of background PFAS concentrations in fish feed used in toxicological experiments. During preliminary experiments to optimize an analytical method for PFAS, background levels were quantified in a commercial fish feed used broadly across zebrafish facilities, and specifically being used for a PFAS dietary exposure in zebrafish. The objective of this study was to optimize and demonstrate an analytical method for quantifying background and overspiked PFAS in commercial fish feed samples.

To evaluate the fish feed from different sources, a variety of commercial fish feed samples were obtained from suppliers in the United States and the European Union. An existing biota extraction method was modified to extract 3 g of fish feed but required two additional steps of clean-up with solid phase extraction. The method was developed for the recovery of 50 targets and 1400 suspect PFAS, and a visualization program (Kendo) was used to screen for suspect PFAS as part of the workflow. One example of fish feed contained a homologous series of perfluoroalkyl carboxylates ranging from C5- C14, perfluorooctane sulfonate (PFOS), and C4, C6, and C8 (FOSA) sulfonamides that ranged in concentration from 0.1 – 3.8 ng/g fish feed). Another example of fish feed also contained perfluoroalkyl carboxylate C9 (PFNA 0.1 ng/g) and perfluorooctane sulfonate (PFOS 0.2 ng/g). The limit of quantification is 0.03 ng/g fish feed). Knowledge about background PFAS concentration in fish feed, and other animal diets, is essential to consider when interprets the results of toxicological studies and specifically dietary exposure studies.

04.13.08 Consistency Across Fine Particulate Matter (PM2.5) Filters: Chemical Composition and Oxidative Potential Differences on the Same Filter

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Exposure to fine particulate matter (PM2.5) has adverse effects on human health such as increasing cardiovascular and respiratory morbidity. The complex chemical mixtures present in PM2.5 contribute to the difficulty in identifying the mechanisms that cause the observed health effects. To conduct analytical and toxicology studies of PM2.5, collected filters are split into sections. This process allows multiple, often destructive, assays to be performed. Our previous research showed chemical composition differences across a PM2.5 filter, and therefore oxidative potential may have similar differences due to redox active metal concentrations. The goal of our study was to determine the validity of splitting filters for use in multiple analyses by assessing differences in chemical composition and oxidative potential within the same filter. Six PM2.5 filter samples collected from urban and rural locations in Arkansas, United States were used. Black carbon concentrations were measured for each sample. Filters were then 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 a whole particle and soluble fraction were prepared. The extracted, whole particle suspensions and soluble fractions were then analyzed with the dithiothreitol (DTT) assay run in triplicate to determine oxidative potential. Inductively coupled plasma mass spectrometry (ICP-MS) will be run on all samples and controls to compare chemical composition of the filter quadrants (n=30). Significant differences in oxidative potential between filter quadrants were not seen in the whole particle suspension and only seen between filter quadrants in one filter when the soluble portion was run. While most filters had no significant differences between quadrants, the range of oxidative potential was unequal across quadrants. Comparisons between urban and rural samples are underway. Elemental analysis for all samples and controls is in progress. Correlation analysis between oxidative potential and elements will be conducted. This work will provide information about the feasibility of splitting PM2.5 filters for multiple analyses on the same sample.

04.13.09 Down-The-Drain Exposure Assessments in Canada and Mexico With iSTREEM®

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The iSTREEM® model (https://www.istreem.org/) integrates the locations of municipal wastewater treatment plants (WWTPs) with a hydrologic network, providing a framework to assess environmental risk in a spatial context. The model has been widely applied for ecological risk assessments of down-the-drain (DtD) chemicals in the U.S. To address growing challenges outside the U.S., the model was recently expanded to include Canada and Mexico by integrating country-specific WWTP infrastructure data with river hydrology from global datasets. WWTPs are a major exposure route for a wide range of DtD chemicals that are treated and discharged to surface water. To include populations not connected to WWTP, the model utilizes a framework to account for wastewater discharged to septic/onsite systems and direct discharge to surface water. The model was applied to predict the environmental concentrations of two DtD chemicals in Canada and Mexico. Country-specific chemical use, along with removals in wastewater and surface water were parameterized for model simulations. Results from the modeling exercise were compared with publicly available monitoring data and published literature. Additional sensitivity analyses were performed to understand the effect of varying model inputs to the results. This work highlights the practical application of the spatially resolved and probabilistic distributions generated by the model as a ready-to-use tool for exposure assessments. The evolution of the iSTREEM® model reflects recent scientific advances in DtD exposure modeling to address current global challenges and needs such as assessment over broad geographies, incorporation of probabilistic variability, spatially explicit distributions, and accessibility of this enhanced utility for end-users.

04.13.10 Ecotoxicological Hazard Screening and Fate and Transport Assessments of ENDS and Oral Tobacco Products

A. Massarsky, A. Bonin, Y.B. Atalay, Cardno; N.R. Binczewski, A.K. Madl, A.D. Jones, Cardno ChemRisk

New tobacco products, such as electronic nicotine delivery systems (ENDS) devices, e-liquids, oral tobacco products, and other tobacco products not marketed prior to 2007, require a pre-market tobacco application (PMTA) and marketing authorization from U.S. Food and Drug Administration (FDA). A statutory mandate of the PMTA is to provide an environmental assessment (EA) in accordance with the National Environmental Policy Act (NEPA). A requirement of the NEPA EA is a quantitative assessment of the potential environmental impacts of the products throughout their life cycle; including manufacturing, use and disposal. Understanding the potential ecotoxicological risks of ingredients and components of the products is critical in evaluating potential environmental impacts. E-liquids and oral tobacco products are complex mixtures typically comprised of nicotine, propylene glycol, vegetable glycerin and a variety of flavorants. While ecotoxicological characteristics of some industrial chemical mixtures have been assessed, to date, no study has evaluated the ecotoxicological characteristics of e-liquid mixtures. A novel approach was developed, in which the ecological structure activity relationships (ECOSAR) program was used to estimate the ecological toxicity of individual chemicals in complex e-liquid mixtures identified in the published literature. Chemicals identified with the highest acute and chronic toxicity to relevant aquatic receptors were considered likely to exhibit the greatest ecological risk. The two chemicals with the highest ecological toxicity selected through the ECOSAR screening process, along with nicotine, propylene glycol, and vegetable glycerine were carried forward into the impact assessment in the EA. Fate and transport of the selected chemicals was characterized using the U.S. Environmental Protection Agency Exposure and Fate Assessment Screening Tool (E-FAST) modeling program under theoretical but low likelihood manufacturing and end-use release scenarios. In addition, a supplemental quantitative modeling approach was used to evaluate the impacts of littering of the product in the urban environment. Estimated concentrations of select chemicals were compared to available ecological toxicity benchmarks to support the impact assessment. The combination of ECOSAR and E-FAST applied to e-liquid, ENDS, and oral tobacco products enabled a rigorous evaluation of potential environmental impacts during the product life cycle.

04.13.12 Evaluating the Reliability of Chemical Exposure Data for Use in Environmental Risk Assessment

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Regulatory monitoring of chemicals in surface waters is routinely undertaken to assess the state of the environment, perform chemical risk assessments, undertake chemical prioritisation exercises, and support legally binding compliance checking. Often these chemical exposure data are compared against Predicted No Effect Concentrations or a Water Quality Guideline. If we follow accepted risk assessment paradigms, we could typically move from using precautionary estimates of chemical exposure concentrations for an activity such as chemical prioritization, through limited datasets of targeted 'spot samples' with sparse spatial and temporal coverage for screening and localized assessments, to comprehensive routine or continuous monitoring for chemical risk and potentially legally binding compliance assessments. Ideally, with greater investment of time and resources chemical exposure datasets should become more environmentally relevant and can be utilized for higher level assessments. It is therefore critical to ensure the chemical exposure data set is relevant for the purpose for which it is to be used. In addition to assessing the relevancy of chemical exposure data, the reliability of the data also requires evaluating. Specifically, reliability of exposure data is principally considered to address the methodological aspects performed post sample collection and the characteristics of the chemical exposure data set and would include aspects such as LoD and LoQ, degree of censoring, identification of the number of different LoQ values, and how they are distributed within the measured data, frequency of sampling and pattern of censored data, etc. In this presentation we outline a decision-making process based upon reliability aspects of chemical exposure datasets, that can support the identification of exposure data that would be fit for the purpose of risk assessment, for example defining a specific percentile value from the data set. This weight of evidence scheme uses simple tests relating to the degree of censorship in the data set, and particularly the importance of censored data in the upper portion of the distribution that is used for deriving the summary statistics that are used for the risk assessment process. We use examples from European-wide Water Framework chemical exposure datasets for amoxicillin, estrone, diclofenac, imidacloprid and thiamethoxam to illustrate how the scheme would function and how the outputs should be interpreted.

04.13.13 First Time Non-Target Screening of Emerging Organic Pollutants in Sediments and Fish from the Eastern Mediterranean Coast: Lebanon

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The overexploitation of coastal resources along the Lebanese coast are constantly increasing. In addition, the coastal area is submitted to various anthropogenic pressures due to the presence of uncontrolled solid waste dumpsites and the discharges of domestic and industrial effluents into the sea without prior treatment. Hence, the objective of the present study was to assess the state of the Lebanese marine environment by determining the occurrence of emerging Persistent Organic Pollutants (POPs) in marine sediments and fish (*Euthynnus alletteratus, Mullus barbatus, Diplodus sargus*) collected along the Lebanese coast during November 2019. Sediments and fish samples were sequentially extracted using a semi-pressurized solvent extraction device (EDGE from CEM), a fat clean-up was applied and then samples were pre-concentrated and screened for semi-volatile organic pollutants such as OCPs, BFR and their metabolites, PCDDs, PCDFs, PAHs and PCBs using firstly single quadrupole GC-EI-MS and as an extension study, preliminary tests were conducted on GC-APCI-tims-TOF HRMS since ion mobility spectrometry (IMS) offers an orthogonal separation dimension based on the ions' size, shape, and charge, known as collisional cross section (CCS). Thus, IMS enhances peak capacity and can be adapted to complex sample matrices. Meanwhile, CCS values provide a further confident identification parameter. For the first time in Lebanon, these contaminants are identified, quantified and reported on the following: extract ion, mass spectrum, and retention time for experiments run by GCMS and exact mass, true isotopic pattern, and Ion Mobility for the parallel appraisal run by GC-tims-TOF. ∑PAHs concentrations in sediments ranged between 15.08 and 2132.88 ng/g dw, while ∑PCBs levels ranged between 0.044 and 32.13 ng/g dw. Various pesticides and herbicides were also detected for the first time in the Lebanese marine environment. Sediments and fish collected near landfills and effluent discharges showed significant concentrations of various contaminants. The results of the present study highlighted the potential contribution of coastal landfills and discharges to the organic pollution. The perspectives and benefit of Non-Target Screening by GC-timsTOF are also promising on Halogenated containing contaminants and their byproducts and more studies are being investigated to ensure the reproducibility of the method and to enrich the in-house compound library.

04.13.14 Forensic Fingerprinting Through Pattern Recognition of a Robust Per- and Polyfluoroalkyl Substance (PFAS) Occurrence Dataset

Z. Neigh, AECOM / Design and Consulting Services; M. Borgens, AECOM / Environmental Science and Management; R. Gwinn, AECOM / Environment

To identify and characterize potential sources, the complex nature of per- and polyfluoroalkyl substance (PFAS) contamination requires a data-rich forensic fingerprinting approach, combining data science applications for pattern recognition with analytical chemistry techniques. We have developed and applied an exploratory, adaptive forensic investigation approach to maximize the information extracted from the data. An exploratory data analysis is first performed on the PFAS compositional data to identify patterns and statistically define the relationships that differentiate the PFAS mixtures. The output of the pattern recognition process is a labeled dataset, where each sample is classified as part of a cluster comprised of similar PFAS mixture signatures using algorithms such as hierarchical clustering. The distinct PFAS mixture signatures represented by each cluster can then be used as a forensic fingerprint. Through further exploration and statistical analysis, the PFAS fingerprints are refined as a basis to test hypotheses and discover associated PFAS sources. This approach leverages existing targeted-PFAS data to create valuable forensic information and can be easily combined with additional lines of forensic evidence such as the presence of non-targeted PFAS indicator compounds.This forensic investigation approach was applied to a large national PFAS occurrence dataset (n > 4,000), curated from more than 30 PFAS investigations performed by AECOM and several publicly available data sources. The samples in this dataset are representative of several different environmental matrices with PFAS contamination associated with a diverse list of potential sources including various aqueous film-forming foam (AFFF) formulations, industrial atmospheric deposition, manufacturing releases, wastewater effluent, and landfills. Through our forensic analysis, the predominant PFAS mixture signatures were identified and statistically defined. The distinct collective PFAS signature represented by each cluster was then used as a forensic fingerprint to explore potential source associations using published PFAS source material information and to evaluate environmental fate and transport behavior. Our findings demonstrate that the labeled dataset resulting from the pattern recognition process can be used to train a predictive classification model and form the basis for a machine learning tool that can associate unknown PFAS compositions with potential sources.

04.13.15 Health Risk From Volatile Organic Compounds in Northwestern US Wildfire Smoke

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Wildfire seasons in the Western US have been getting more severe, bringing with them a decrease in regional air quality during summer months. Particulate matter is most commonly measured in various locations yet ground level volatile organic compound (VOC) data are more sparse. We measured about 100 VOCs in various fires in Idaho and Washington using sorbent tubes and thermal desorption-gas chromatography-mass spectrometry. Benzene in particular was used in estimation of cancer risk assessment for populations close to these fires. Human health risk increased up to ten-fold due to wildfire smoke and projections were made for higher frequency wildfire events.

04.13.16 Increasing the Availability of Sampling Bias Factors for Pesticides With Sparse Monitoring Data

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The U.S. Environmental Protection Agency's Office of Pesticide Programs uses pesticide monitoring data in concert with computer modeling to estimate pesticide concentrations in aquatic environments. Waters are generally sampled infrequently, which leads to uncertainty in whether the samples are representative of upper-bound pesticide concentrations. To avoid underestimating pesticide concentrations in risk assessments, SEAWAVE-QEX is used to estimate daily pesticide concentrations from sparse monitoring data using streamflow as a covariate. The output chemographs from SEAWAVE-QEX may be used to develop sampling bias factors (SBFs), which can be used to estimate upper-bound concentrations at other monitoring sites where data were not suitable for input into SEAWAVE-QEX. Previous SBF development efforts focused on four pesticides – atrazine, chlorpyrifos, carbaryl, and fipronil. The present work updates the currently available SBFs for these four pesticides by improving the SEAWAVE-QEX fits used to generate daily pesticide chemographs and expands the number of pesticides that SBFs are available for to encompass a greater number of pesticides with varying fate, transport, and use profiles.

04.13.17 Inhalation Exposure Assessment of Chemicals in Products Available to Consumers Under the Chemicals Management Plan: A Retrospective Analysis

K. Himmat, Health Canada

The Chemicals Management Plan (CMP) is a Government of Canada initiative aimed at reducing the risks posed by chemicals to Canadians and their environment. In order to determine the potential human health risk posed by these chemicals, the potential exposure of Canadians from all applicable sources and uses (based on available information) is considered for every relevant route of exposure. This poster will focus on the inhalation route of exposure and present a retrospective analysis of inhalation exposure scenarios for products available to consumers described in a number of CMP screening assessments published by the Government of Canada over the last decade. Inhalation exposure scenarios included in the retrospective analysis represent the use of a wide variety of products (e.g., hair styling products, spray paints, and aerosol lubricants) containing priority substances assessed under the CMP. Multiple factors such as product type, assumptions with respect to exposure duration or frequency of use of a product, as well as the exposure model applied have an impact on the ultimate outcome of the exposure assessment. Inhalation was found to be a driver of exposure for many chemical substances assessed under the CMP, and the results of this retrospective analysis demonstrate the importance of this route of exposure in chemical risk assessment.

04.13.18 Long-Term Monitoring of Sediment Surrounding Creosote-Treated Piles in a Sensitive Marine Environment in Sooke, British Columbia, Canada

P. Mcmanus, Fisheries and Oceans Canada

Creosote-treated piles, despite long-term adverse impacts on the environment, are widely used as a component of infrastructure in marine settings. Creosote-treated wood is durable and cost efficient but introduce a source of polycyclic aromatic hydrocarbons (PAHs) into the environment when used as a building material. Differentiating the contribution of creosote-treated beneficial use structures (ie/ wharfs or docks) as a source of PAHs at small craft harbours from other potential PAH sources is essential in addressing uncertainty during site assessments. In 1995 Fisheries and Oceans Canada began a pilot study off the coast of Sooke, British Columbia, Canada to provide a scientific basis for establishing guidelines on the use and placement of creosote treated wood in sensitive marine aquatic habitats. Creosote-treated sets of piles (dolphins) were installed in an undisturbed location, sediment chemistry, toxicity and benthic infaunal community structure were then monitored periodically until 2005 whereupon the piles remained but sampling events ceased. The results of this study were the basis for assessment policy and site management decisions for all small craft harbors in Canada under federal government custody. In 2020 the site was revisited, inspected and the sediment surrounding the piles were resampled. Detected PAHs form a spatial gradient that has been mitigated over time. These results will be used to inform future guidance around creosote-treated timber used in infrastructure in marine environments.

04.13.19 Method Development for the Isolation and Determination of Prymnesin Toxins in Ambient Water

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Prymnesium parvum is an invasive algal species that forms harmful algal blooms (HABs) in inland environments and is responsible for devastating fish kills causing ecological and economic damage. While progress has been made in developing approaches to manage *P. parvum* blooms, many knowledge gaps and research needs remain. Much uncertainty remains in identifying toxic chemicals produced by *P. parvum* and under what environmental conditions those toxins are produced. The lack of commercially available standards complicates the development of a quantitative mass spectrometric method for prymnesin toxins. Toxins must be harvested from *P. parvum* and then isolated for use as analytical reference materials.While blooms of *P. parvum* have been documented in the eastern hemisphere since the early 1900s, blooms in the western hemisphere only started in the mid-1980s, and then only localized in the south-central USA. The first confirmed blooms of *P. parvum* in North America were identified in Texas in 1985 on the Pecos River. Rapid proliferation of blooms in the western hemisphere occurred during the early 2000s, with reports happening in 23 states, including Alabama, Arizona, Arkansas, California, Washington, Hawaii, New Mexico, Wyoming, North Carolina, South Carolina, Florida, and Georgia. Blooms now occur in all southern regions of the USA and some northern regions. The USEPA has taken steps to improve the analytical methodology available for the detection and quantification of HABs. Data will be presented describing the isolation and purification of two prymnesin toxins from a *P. parvum* culture (UTEX LB 2797, Texas) to be used as reference standards. *P. parvum* cultures were conducted at 20^{o}C, 16h light:8h dark, under a light intensity of 20 µmol/m 2 /s using a F/2 media in artificial sea water. The standards will be used to develop a quantitative procedure to measure the prymnesin toxins in ambient water using solid phase extraction (SPE) with liquid chromatography/quadrupole time-of-flight mass spectrometry (LC/QTOFMS). Precision and accuracy data for various SPE sorbents and preservative combinations in deionized and ambient waters will be presented. Stability test results for primary dilution standards, liquid chromatography parameters, and results of preliminary aqueous and extract holding time studies will be shown.

04.13.20 Organic Contamination of Canadian Lakes: Is 'Pristine' a Thing of the Past?

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Trace organic contaminants (TrOCs) are mostly studied in drinking waters, rivers, and wastewater, but data is lacking regarding their presence in lakes. For that reason, a multi-residue method was developed to quantify 51 TrOCs in lake water. This method was applied to evaluate the contamination of 295 lakes sampled across Canada in a stratified random design with ecozones, size, and human activities in their watersheds as strata. This includes many remote lakes with limited access. The purpose of this work was to fill the gap of knowledge on lakes' contamination in Canada. The TrOCs analyzed in this study include regulated organic pollutants, such as pesticides, as well as contaminants of emerging concern, such as pharmaceuticals, personal care products and consumer products additives. The compounds were extracted by solid phase extraction (SPE) and analyzed using ultra performance liquid chromatography coupled to triple quadrupole mass spectrometry (UPLC-QqQMS). At least one contaminant was detected in 84% of the lakes and up to 16 TrOCs were detected per lake. The most frequently encountered compounds were diethyltoluamide (DEET), tris(2-chloroethyl) phosphate (TCEP) and caffeine, each present in more than a third of the lakes. Concentrations spanned from 0.4 ng L^{-1} to 18 µg L^{-1}. Nine TrOCs from various sources had concentrations over 1 µg L^{-1}. Pesticides and pharmaceuticals had similar frequencies of detection with each category found in about 50% of the lakes. Moreover, 8% of lakes exceeded 100 ng L^{-1} for a single pesticide and 2% exceeded 500 ng $L^{-1}$for the sum. As for pharmaceuticals, 8% of the lakes had concentrations over 100 ng L^{-1} for a single compound and 3% over 500 ng L^{-1} for the sum. However, their distributions were dissimilar and were linked to the type and extent of land use on lakes' watersheds: pesticide concentrations increased with the amount of agriculture around a lake, while pharmaceuticals were detected mostly in urbanized watersheds. More specific factors are being examined to explain the occurrence of each contaminant class in lake water. The results of this work represent the first reference point to monitor the evolution of the contamination of Canadian lakes by TrOCs and demonstrate that a high proportion of the sampled lakes show a significant anthropogenic chemical footprint.

04.13.21 Phthalates and Phthalate Alternatives Analysis Using Gas Chromatography Mass Spectrometry for Wristband and Environmental Passive Sampling Matrices

K. Adams, R. Scott, C. Haggerty, K.A. Anderson, Oregon State University / Environmental and Molecular Toxicology

The demand for phthalate plasticizers has accelerated due to the discovery of their unique properties that were advantageous to a wide array of everyday consumer items. With a global production of six million tons per year has caused more awareness of the environmental health impacts and contamination of phthalates within the last thirty years. This drove many countries to implement various regulations for particular phthalates from products such as toys to industrial emission policies. A fast Gas Chromatography Mass Spectrometry (GC-MS) method for phthalates was developed to quantitate traditional and current phthalates. The method is applicable for over 30 phthalates and includes common alternative (non-phthalate) plasticizers, which was established using an Agilent 8890 GC 5977B MS. The target compounds utilized both scan and selected ion monitoring (SIM) modes. The analytic method validation included inter and intraday variability, demonstration of calibration with an r2 value of at least 0.98 or better, as well as determination of limits of detection and quantitation. The validation process included assessment of both silicone wristband passive samplers and low density polyethylene (LDPE).

04.13.22 Probability-Based Assessments of Per- and Polyfluoroalkyl Substances (PFAS) in Great Lakes Fish

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Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic chemicals used to make consumer and industrial products resist oil, grease, stains, and water. Their unique physical and chemical properties prompted their use in non-stick cookware, waterproof clothing, fabric stain protection, and firefighting foams. PFAS are of concern because they are persistent, bioaccumulative, and toxic chemicals, they are broadly distributed in the environment, and they have been shown to accumulate in fish. Animal studies show that increased PFAS exposure may pose health risks that can affect development, reproduction, and immune function. In response to increasing concerns about potential PFAS exposure and health impacts, the U.S. Environmental Protection Agency (EPA) conducted studies of PFAS in Great Lakes fish using the unequal probability design (i.e., a Generalized Random Tessellation Stratified (GRTS) survey design) developed for the Agency's National Coastal Condition Assessments. The GRTS survey design was stratified by Great Lake and country (limited to U.S. waters), and the unequal probability of selection was based on the proportion of shoreline length in each of the eight Great Lakes states. The nearshore zone was defined as the region from the shoreline to a depth of 30 m or to a maximum distance of 5 km from the shoreline, and sampling sites within the nearshore zone polygons were randomly selected in the five Great Lakes. Fish species commonly consumed by humans were collected from 157 and 152 randomly selected U.S. nearshore locations throughout the Great Lakes for the 2010 Great Lakes Human Health Fish Tissue Study and the 2015 Great Lakes Human Health Fish Fillet Tissue Study, respectively. Fillet composite samples were analyzed for 13 PFAS using high performance liquid chromatography with tandem mass spectrometry. Perfluorooctane sulfonate (PFOS) dominated in frequency of occurrence and was detected in 100% of all samples. PFOS concentrations ranged from 1.9 to 80.0 ng/g in 2010 and 0.5 to 64.4 ng/g in 2015. Cumulative distribution functions were developed to quantify PFOS concentrations in fish fillet composites compared to the sampled population area, which was defined as 11,091 km^2 for the 2010 study and 16,843 km^2 for the 2015 study.

04.20 Late Breaking Science: Chemistry and Exposure Assessment

04.20.01 A Review of Organic Micropollutants Photodegradation Process to Develop a QSAR Model

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The amount of anthropic chemicals, such as pharmaceuticals or pesticides, designed to improve our way of life is increasing in various surface waters. Either through the discharge of wastewater effluents or runoff, a part of these chemicals reaches local surface waters where they are subsequently referred to as micropollutants. The fate (i.e. adsorption on particulate matter, bioaccumulation, biotic and abiotic degradation/ transformation...) of these micropollutants in river water is a major environmental concern and their vulnerability to photodegradation should be assessed. On the other hand, laboratory experiments do not represent the best way to assess the photodegradation kinetics in real environmental conditions and cannot be performed on each molecule to identify the corresponding half-life. Therefore, the objectives of this study were to: (1) examine the available bibliography on organic micropollutants photodegradation related to QSAR models and molecular descriptors (MDs), (2) to find the suitable QSAR model in our dataset, and (3) to define the gap and the research needs. We gathered the articles retrieved on SCOPUS and Web of Science since the 2000s focusing on molecules with their MDs, environmental parameters, methods used to perform QSAR model, etc. Our dataset is composed by 33 micropollutants (whose MDs values and photodegradation QSAR models available in the bibliography are scarce), with their half-life values and 40 molecular descriptors collected from the database attached to the TyPol classification tool. Then, a classification and regression tree (CART) was used to link molecular descriptors to water photodegradation half-life measured by our laboratory. The model was built using 70% of the micropollutants and subsequently validated using the remaining 30%. Preliminary results using CART model revealed that the five most relevant molecular descriptors to assess the photodegradation half-life were respectively the number of multiple bonds, the number of oxygen atoms, the number of circuits, the number of rotatable bonds, and the connectivity index of order 3. According to this model, the micropollutants were split into six groups with half-life ranging from 8.2 hours to 81 hours. This model has an R^2 of 0.61 and a root mean square error (RMSE) of 22.2 hours. These first results show that knowing only the structure of a compound, it is possible to assess its half-life under photodegradation in surface water.

04.20.03 Application of a Quality Scoring System for Assessing Per- and Polyfluoroalkyl Substances (PFAS) in Organic Solvents for In Vitro Toxicokinetic Testing

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PFAS represent a large chemical class for which limited toxicity and toxicokinetic information is available. Given the persistent and bioaccumulative properties for legacy PFAS, category or grouping strategies are being employed using *in vitro* new approach methodologies (NAMs) to evaluate the larger PFAS space. One critical aspect to ensuring accurate and complete evaluation of PFAS is the quality of the material being assessed. Numerous organic solvents, including dimethyl sulfoxide (DMSO) and ethanol, are used as diluents for *in vitro* evaluation. Using mass spectrometry approaches, a quality scoring system was created to provide a simple pass or fail notation with descriptive flags for each PFAS stock by examining for parent mass and impurities. 201 unique PFAS prepared in 469 stock solutions were assessed through this scoring system, where 75 unique PFAS were investigated by liquid chromatography mass spectrometry (LC-MS) and 126 were characterized by gas chromatography mass spectrometry (GC-MS). Most failed LC-MS examined PFAS belonged to the hexafluoropropylene oxide acid family in DMSO, including GenX, while 60 GC-MS amenable PFAS with predicted low boiling points were discarded due to failure to detect during analysis. Failed stocks have been removed from use in any EPA *in vitro* evaluations. Ultimately, over 115 PFAS in DMSO stocks received passing quality scores to allow for further *in vitro* testing. *In vitro* measures of PFAS plasma protein binding (PPB), a toxicokinetic property used to inform PFAS bioaccumulative potential, were generated across the LC-MS PFAS DMSO stocks. PPB measures by ultracentrifugation showed very high binding rates [i.e., fraction unbound (F_u) = 0.001for perfluorooctanoic acid] across 75% of PFAS examined. Six PFAS with high predicted water solubility exhibited less protein binding, with F_u values ≥0.10, including hexafluoroglutaric acid (F_u = 0.53). For perfluoroalkyl carboxylic acids, increasing chain length exhibited higher PPB (C3: perfluoropropanoic acid F_u = 0.31; C10: perfluorodecanoic acid F_u = 0.003). This stock quality scoring system provides an informed guide on how to proceed with performing *in* vitro experiments to advise NAM-anchored, risk-based assessment of PFAS. *The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the USEPA.*

04.20.04 Application of Ft-Icr Mass Spectrometry to Identify Emerging Contaminants

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Molecular-level identification of emerging contaminants in a range of environmental systems requires advanced analytical techniques that can address the complexity associated with real-world environmental samples matrices (e.g., marine and terrestrial waters, soils, sediments, and waste water). Ultrahigh resolution FT-ICR MS is ideal for molecular characterization of biochar and soil organic matter, where tens of thousands of unique elemental compositions are routinely resolved and assigned with high mass measurement accuracy. Ultrahigh resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) routinely achieves resolving power sufficient to address the complexity of the sample matrix for field samples, and identify novel emerging contaminants at unprecedented detail. Here, we will highlight several applications of FT-ICR MS that identify emerging contaminants from microplastics, biochar, oil degradation, asphalt runoff, and PFAS (per- and polyfluoroalkyl substances). Several specific applications will be highlighted include biochar and microplastics. Biochar is the carbonaceous residue formed through forest fires or biomass pyrolysis, and its deposition in soils through natural burning or as an agricultural amendment has gained notable attention worldwide. Addition of biochar to depleted soils increases organic soil carbon and also restrains the growth of atmospheric CO2 through soil carbon sequestration. Charred biomass also contains toxic trace metals and recalcitrant, persistent organic pollutants, such as polycyclic aromatic hydrocarbons (PAHs). Sunlight exposure is a control of long-term plastic fate in the environment that converts plastic into oxygenated products spanning the polymer, dissolved, and gas phases. However, our understanding of how plastic formulation influences the amount and composition of these photoproducts remains incomplete. Here, we characterized the initial formulations and resulting dissolved photoproducts of four single-use consumer polyethylene (PE) bags from major retailers and one pure PE film. Consumer PE bags contained 15–36% inorganic additives, primarily calcium carbonate (13–34%) and titanium dioxide (TiO2; 1–2%). Sunlight exposure consistently increased production of dissolved organic carbon (DOC) relative to leaching in the dark (3- to 80-fold). All consumer PE bags produced more DOC during sunlight exposure than the pure PE (1.2- to 2.0-fold).

04.20.05 Are Beer Cans a Potential Source of Microplastic and Chemical Additives in the Marine Environment?

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Nowadays, marine plastic pollution has drawn public attention. In addition, high occurrence of metal debris has also been reported in some studies, yet less concern has been given to this anthropogenic waste. Polymer coating utilized in metal packaging (as a boundary between metal and products) may be a potential source of microplastic pollution as it enters the environment and disintegrates. This study investigates the polymer coating used for both lid and body as well as inside and outside layer of 27 beer cans from 16 countries. No correlation found between polymer type used and its origin countries. Epoxy resin was the most used polymer in both lid (inside and outside) and body (inside). As for the outside layer of can body, poly(1,2-butanediol isophthalate) was frequently found to be used. Furthermore, plastic additives analysis was also performed to understand its environmental risk. Plasticizer of bis (2-ethylhexyl) phthalate (DEHP) was the major additives used, followed by antioxidant of butylated hydroxytoluene (BHT) with the concentration as high as 5,300 ng/g and 520 ng/g respectively. Plasticizer of dioctyl adipate (DOA) was hardly detected in samples. However, maximum of 9,600 ng/g of DOA was detected in Belgian beer can. There was no particular pattern of relation between country origins and plastic additives contained. In addition, one environmental sample was found to be heavily broken down. There was hardly outer coating remained, while the inner coating was still intact and being peeled out. Moreover, this aging can contained more phthalates plasticizer as well as higher concentration of chemical additives for one to two orders of magnitudes compared to the brand-new can. This suggests that metal debris may be a potential source of microplastic chemical additives pollution as it disintegrate in the environment.

04.20.06 Assessing Silicone Wristbands As Sampling Devices by Determining Pesticide Recovery

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Silicone wristbands are comprised of a porous surface with the potential to sequester organic contaminants in the environment. Their properties allow for them to be used as a novel sampling approach to assessing personal human exposure to environmental contaminants. The purpose of the study was to understand the effectiveness of silicone wristbands as sampling devices. This was addressed by identifying and quantifying pesticide recovery from exposed wristbands. Triplicate groups of wristbands were dosed with 37 persistent organochlorine or organophosphate pesticides and then extracted to estimate human exposure through recovery. Results suggest that silicone wristbands have the potential to absorb a number of pesticides and organic contaminants, although at varying rates and quantities. As more uptake and sequestration rates can be established, wristbands have the potential to serve as indicators of human exposure to a variety of pesticides and other chemical groups at trace amounts.

04.20.07 Development of a New Evaporation Exposure Model: Chemical Product Evaporation Model (CPEM)

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Existing evaporation exposure models, such as the ConsExpo model, are used to estimate chemical concentrations under the assumption that the volume of the product is constant or that the concentration of a target substance in the product is constant. The volume of a product decreases during use, and it is expected that the concentrations of substances within consumer products will also change over time. To explain the effect of the change in product volume, the ‘Chemical Product Evaporation Model (CPEM)’ was developed. First, it was theoretically and experimentally demonstrated that the volume of a product decreases linearly when the surface area of the product is invariant. The product volume was added to the CPEM as a state variable and then incorporated into the ConsExpo evaporation model. If the saturation kinetics were not considered in the CPEM, the concentration of a target substance in the air was predicted to be 2% higher than that predicted by the CPEM considering saturation kinetics. From the CPEM without saturation kinetics, a criterion was derived to determine whether the substance concentration in a product increased or decreased. The substance concentrations in various products were found to have increased in 65% of 4,600 pairs of 20 consumer products and 230 substances. In conclusion, because the ConsExpo model underestimates the exposure concentration in air due to evaporation, it should be replaced by the CPEM to determine screening levels in exposure assessments. If the substance concentration in a product decreases, the average concentration of a target substance in the air can be estimated simply from the initial substance concentration in the product and the product volume reduction rate. If the substance concentration in the product increases, it is recommended to use the CPEM without saturation kinetics for screening purposes.Acknowledgement: This work was supported by Korea Environment Industry & Technology Institute(KEITI) through Technology Development Project for Safety Management of Household Chemical Products □Development of the technology to assess the environmental pollutant load and exposure of hazardous substances contained in products□, funded by Korea Ministry of Environment(MOE) (2020002970003, 1485017539)

04.20.08 Environmental Contaminants from Hydraulic Fracturing Flowback Water from the Duvernay Formation at Two Different Locations in Alberta, Canada

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Chemical characterization of hydraulic fracturing (HF) flowback and produced water (FPW) is important to the economic and environmental sustainability of HF operations. Improved understanding of FPW water chemistry allows for better decisions surrounding water re-use, as well any environmental implications should a spill of produced water occur. In this research, FPW samples were collected from the Fox Creek and Three Hills regions of Alberta, Canada. Samples were analyzed for major cations and anions, trace elements, total organic carbon and water isotopes ($\delta^{18}O$ and $\delta^{2}H$). High resolution mass spectrometry was also conducted to identify organic contaminants from FPW. Multivariate analysis was applied to discover correlations among different water chemistry parameters. Principal component analysis and hierarchical clustering were used to identify groupings observed at two different geographic locations as well as throughout the flowback period at Three Hills. Saturation indices of the minerals in the FPW samples were calculated. Overall, samples analyzed from the two regions were similar in chemical composition but showed differences in some of the lower abundance dissolved elements and trace organic compounds. Polymers and quaternary ammonium compounds have been identified in the FPW. Additionally, water chemistry from a single well over the 7-month sampling period showed increasing concentrations of Ca and Mg, and a decreasing concentration of SO_4. Several trace elements also showed trends of either increasing or decreasing concentrations over time. The results provide important information for assessing the reusability of FPW and guidance for wastewater management. While reuse of FPW is desirable, it is not feasible in many operations. In these scenarios, FPW transportation for offsite treatment and/or disposal increases the risk of an environmental spill. This work provides knowledge to aid onsite clean-up efforts and evaluate the environmental fate and toxicity of FPW contaminants.

04.20.09 EPA Trash Free Waters: Directions in Microplastics Research

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As part of its national program to prevent trash from entering waterways, the U.S. Environmental Protection Agency (EPA) Trash Free Waters Program convened an expert workshop in June 2017 to identify and prioritize the scientific information needed to understand the risks posed by microplastics. Workshop participants discussed the state of the science on analytical methods, environmental sources, and the toxicology of exposure to microplastics. Participants developed conceptual models that describe exposure pathways and impacts of microplastics to environmental and human health. In 2021, the Program held discussions with subject matter experts and conducted a targeted literature review to update the previous workshop findings and identify information gaps and emerging areas of interest within microplastics research. The conclusions were compiled into a report to assist the scientific research and funding communities, and spur momentum toward solutions that reduce and prevent microplastics at their source. This presentation will review the identified priority research needs for the following topics: analytical methods to measure microplastics; source, transport, and fate studies to understand how microplastics move within environmental compartments;

environmental assessments to better characterize toxicity using ecologically relevant study designs; and human health assessments to begin characterizing exposure and impacts to human populations.

04.20.10 In Vitro Acute Toxicity Assessment of Tire Tread Particles Using Rainbow Trout Cell Lines of Gill and Intestine

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Tire and road wear particles (TRWP) are generated by the abrasion of tires while driving, and recent questions were raised in regards to their potential contribution to microplastics released into the aquatic environment and their potential toxicological impacts. Our study aimed to determine 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 (RTgut-GC) epithelium. Acute exposure to cryogenically milled tire tread (CMTT) particles and CMTT digestate following *in vitro* digestion was investigated. To obtain the effective concentrations (EC50) causing a 50% cell viability loss, tests were performed in accordance with OECD TG249: cell viability was assessed after 24 hours exposure using a multiple-endpoint assay indicative of cell metabolic activity, membrane integrity and lysosome integrity. Finally, chemical composition of the exposure medium was analyzed to assess which chemicals could be responsible for the observed acute effects. Our results show that metabolic activity was the most sensitive indicator of CMTT and CMTT digestate toxicity. EC50 values after direct exposure to RTgill-W1 and RTgutGC cells were estimated at 2.01 g/L and 3.80 g/L, respectively. 2-Mercaptobenzothiazole was found at the highest concentration among organic chemicals leaching out from the particles. It could be a major contributor to the loss in metabolic activity based on its toxicity to rainbow trout *in vivo*, although additional particle or mixture effects cannot be ruled out at this point. For digestate toxicity, EC50 was determined at 12.15 g of tire particle/L of digestive fluid. The observed toxicity could be due to high amounts of Zn, N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine (6PPD), 1,3-Diphenylguanidine (DPG) or 2-Mercaptobenzothiazole leaching out during *in vitro* digestion. Traces of 6PPD-quinone were also detected in the exposure medium, but additional cytotoxicity assays using the RTgill-W1 cell line showed no significant toxicity of this compound up to 3 mg/L. Overall, our *in vitro* toxicity data agree with reports of acute fish *(in vivo)* toxicity and show that acutely toxic concentrations of CMTT are well above concentrations measured in river water (0.3 to 4 mg/L). However, we determined the highest no-observed-effect concentration at 10 mg/L, while up to 100 mg/L were measured in road runoffs.

04.20.11 Neonicotinoids and Diamides in Canadian Prairie Streams

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Neonicotinoid and diamide insecticides are used extensively in Canadian agriculture, yet despite their high water solubility the occurrence and concentrations in Canadian streams and rivers is understudied. We report on the concentration of ten neonicotinoids and two diamides in our study of 16 lower order streams in south-central Saskatchewan, Canada from 2017-2019. Analysis for seven neonicotinoids occurred in all three years of the study, while analysis for three additional neonicotinoids and two diamides were included from 2018-2019. About 45% (177 samples) of all water samples collected (392) had detections of at least one of seven continuously monitored neonicotinoids present, but compounds and concentrations varied annually and varied substantially among subwatersheds. Likewise, between 2018 and 2019 (256 samples), at least one of the 12 insecticides were detected half the time (129). Overall, thiamethoxam was the most frequently detected neonicotinoid, followed by clothianidin and imidacloprid. Runoff from snowmelt and rainfall had significantly higher concentrations of thiamethoxam and clothianidin compared to the baseflow condition, yet were similar to each other. Imidacloprid concentrations were similar across event types with most detects occurring during rainfall events. Generally, insecticide concentrations were associated with larger areas of canola and cereal crops in the subwatersheds. Some sites had clear concentration patterns, such as Spirit Creek, which had high concentrations of neonicotinoids and frequent detections of chlorantraniliprole during periods of higher flows, likely reflecting the more intensive agriculture practices in the watershed. Insecticide mixtures primarily consisted of the three most commonly used neonicotinoids: thiamethoxam, clothianidin, and imidacloprid. Between 2018 and 2019, roughly 15% of samples (40) had detectible insecticide mixtures that included either chlorantraniliprole or cyantraniliprole diamides where the majority of those samples (39) included at least one neonicotinoid, highlighting the increasing usage of diamides in Canada. High variability in these insecticide detections, concentrations, and mixtures point towards the difficulty in assessing the risk of these compounds to aquatic ecology. Overall neonicotinoid and diamide insecticides were found to be present throughout the year in many Saskatchewan prairie streams, with detections changing year-to-year and across sites. The dynamic nature of these insecticides in streams reflects the changes in crops/crop rotation practices, precipitation, prairie hydrology, agricultural practices and environmental conditions.

04.20.12 Temporal Trend of Microplastic and Its Additive Chemicals in Groundwater From Japan: Implication for the Potential Source

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In recent years, there has been increasing concerns about environmental pollution by plastic waste and microplastics (MPs). The occurrence of MPs have been reported in the aquatic ecosystem, but little information is available on MPs and its additive chemicals in groundwater samples. This study investigated the abundance and polymer type of MPs in groundwater from Kumamoto, western Japan and its temporal variation. In experiment, a sample of 100 L groundwater was filtered at the sampling site using a 100 µm mesh nylon filter. The pieces recovered by the filter were treated with hydrogen peroxide solution for degradation of organic material, and solid particle/film/fragment on the filter were picked up for FT-IR analysis. The MPs were detected in groundwater sample at median abundance of 0.9 pieces/100 L ($n = 30$). Polyethylene and poly(vinyl chloride-co-vinylidene chloride) were dominant polymer in groundwater, accounting for 60% to total amount of MPs. Interestingly, temporal variation of MPs abundance was identified in a sampling site; the numbers drastically increased in summer. Pyrolysis-GC/MS was used to analyze additive chemicals in MPs in groundwater, and several plasticizers and lubricants were detected. The potential source of MPs in groundwater have been examined by comparing chemical composition of plastic additives between MPs and plastic products around the sampling site. To our knowledge, this is a first report to identify MPs in groundwater in Japan.

04.20.15 Wide Distribution of Per- and Polyfluoroalkyl Substances in Raw and Drinking Water in Osaka, Japan

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In Japan, perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) in drinking water are regulated by the Ministry of Health, Labour and Welfare, and the regulatory threshold of PFOS+PFOA is 50 ng/L. However, other per- and polyfluoroalkyl substances (PFAS) have no regulatory thresholds, and there is no monitoring data of PFAS in drinking water in Japan. We analyzed 32 PFAS in raw and drinking water in Osaka, Japan. Osaka is located in central Japan, and is one of the largest industrial cities with a population of approximately 8.8

million. Water samples were obtained from 22 water purification plants in Osaka from July 2020 to January 2021. Water samples were extracted by solid-phase extraction, and all PFAS were quantified using a high performance liquid chromatograph-tandem mass spectrometer. The limits of quantification (LOQ) were 0.089–1.0 ng/L, and the recoveries of 16 internal standards varied from 76 to 109%. We detected 15 PFAS such as PFOS (maximum concentration: 9.0 ng/L), PFOA (29 ng/L), PFBS (2.1 ng/L), PFHxS (2.2 ng/L), PFBA (16 ng/L), PFPeA (9.6 ng/L), PFHxA (20 ng/L), PFHpA (14 ng/L), and PFNA (11 ng/L) in drinking water. The concentrations of PFOS and PFOA in this study were lower than those in 2006. We also found HFPO-DA (0.39 ng/L) and 6:2FTS (0.14 ng/L), which have not been previously found in Japan. Our results indicated that many types of PFAS were also distributed in drinking water in Japan. The concentrations of various long-chain PFAS, such as PFNS, PFDS, PFDoS, PFDoA, PFTrDA, PFTeDA, PFHxDA, and PFODA, were below the LOQ. HFPO-DA and PFHxA are known to be alternatives to PFOA. Since the manufacture of PFOA has been regulated by Japanese law since October 2021, it is expected that the amount of these alternatives will increase. These concentrations of these alternatives in drinking water should be continuously monitored. PFAS concentrations in drinking water were similar to those in raw water. This indicated that the removal efficiency of PFAS by existing water treatment methods in Japan, including coagulation-sedimentation process, ozonation, and activated carbon treatment, are low. Although concentrations of PFOS+PFOA in all samples were below the regulation value in Japan (50 ng/L), several types of PFAS were detected in drinking water. Therefore, it is necessary to gather information on the toxicity of these compounds to assess their risk to human health.

05.01 Advancing Endangered Species Risk Assessment and Mitigation from National-Scale to Species-Centric Assessments Using "Best Available" Data

05.01.01 Uncertainties in Predicting the Future Using the Past: Evaluating 20 Years of Temporal Variation in the Use of Pesticides in California

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Under the Endangered Species Act (ESA), assessing whether a federal action jeopardizes the continued existence of an ESA-listed species requires consideration of the duration of the action. In the case of the registration of pesticides by the Environmental Protection Agency (EPA) under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), this is a 15-year period. While pesticide labels identify where and how pesticides may be applied (e.g. which crops may be treated), there is uncertainty where and how treatment will occur over the duration of the action (e.g. the specific locations that will be treated). While estimates on usage may inform where pesticide applications have occurred, they are historic in nature. The extent to which pesticide labels or usage data can represent future use is an important source of uncertainty in assessing the risks posed to ESA-listed species by pesticide registrations. To evaluate the degree to which pesticide use can change over a long period of time, National Marine Fisheries Service (NMFS) evaluated California Pesticide Use Reporting (PUR) data for 1990 to 2017 (the most current year available). California PUR data is publicly available and represents the most comprehensive, reliable usage information available as the state requires reporting for all agricultural applications, and all applications by certified applicators, in California. NMFS selected 153 pesticides and 248 use sites to examine. All possible combinations of a pesticide and a site were assessed, but only those with at least 20 years of data were analyzed. A total of 3269 site:pesticide combinations met these criteria. NMFS found that application of a pesticide to a site can vary quite dramatically over a 20-yr period and in different ways. Several metrics were used to assess changes in pesticide use over the 20 years of PUR data across all the site:pesticide combinations. For example, comparisons were made of the average extent of use between different time periods. For 36.5% of the combinations there was a significant difference in the mean of the later 15-yr period relative to the first 5-yr period. These results highlight the uncertainty introduced in relying on recent historic usage to predict future usage. Addressing this uncertainty is a necessary part of considering the risk of pesticide registrations to ESA-listed species.

05.01.02 A Multi-Scale Probabilistic Approach for the Identification of Potential Pesticide Use Sites for Ecological Risk Assessments

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Ecological risk assessment (ERA) requires estimating the overlap between chemical and receptor distribution to evaluate the impacts of exposure on nontarget organisms. Pesticide use estimation at field level is prone to error due to inconsistencies between ground-reporting and geospatial data coverage; attempts to rectify these inconsistencies have been limited in approach and rarely scaled to multiple crop types. We built upon a previously developed Bayesian approach to combine multiple crop types for a probabilistic determination of field-crop assignments and to examine co-occurrence of critical vernal pool habitats and bifenthrin application within a 5-county area in California (Madera, Merced, Sacramento, San Joaquin, and Stanislaus counties). We incorporated a multi-scale repeated sampling approach with an area constraint to both improve the delineation of field boundaries and better capture variability in crop assignments and rotation schemes. After comparing the accuracy of the spatial probabilistic approach to USDA Census of Agriculture crop acreage data, we found our approach allows more specificity in the combination of crop types represented by the potential application area and improves acreage estimates when compared to traditional deterministic approaches. In addition, our multi-scale sampling scheme improved spatial autocorrelation at finer scales and allowed for estimates of crop rotations that were previously uncaptured. Our approach could be leveraged for more realistic, spatially resolved exposure and effects models both in and outside of California.

05.01.03 Automated Probabilistic Co-Occurrence Assessments for Federally Listed Species

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Section 7 of the Endangered Species Act requires the US Environmental Protection Agency (EPA) to consult with the US Fish and Wildlife Service (FWS) about potential pesticide impacts to federally listed species. Consultation is challenged by the abundance of registered products and listed species, as well as by lack of consensus on best available species distribution data and co-occurrence prediction methods. Our previous work demonstrates that probabilistic estimates of species' ranges and pesticide use patterns improve these estimates. Here we demonstrate a freely available software tool and database that can be used to efficiently generate batches of probabilistic maps and statistical summaries of species distributions, pesticide use, and co-occurrence between the two. To generate probabilistic pesticide use footprints the user first specifies crop application rates which are used in conjunction with county level summaries of USDA Cropland Data Layers to calculate maximum potential usage by year and by county, USDA Crop Reporting District, or state. The user may then provide their own pesticide use data, or use the state level USGS ePest pesticide use estimates provided in the database, to calculate the Percent Crop Treated (PCT). The PCT figures are then multiplied by probabilistic crop footprint rasters and combined to produce the probabilistic pesticide use footprint rasters. The tool uses Maxent software to generate species distribution models (SDMs) based on occurrence records and gridded models of bioclimatic, geographic, and other predictor variables. SDMs are constructed via an iterative model selection procedure that controls for known sources of error such as spatial autocorrelation and prioritizes model fit. The pesticide use footprint and SDMs are then multiplied to compute probability of co-occurrence, which is summarized within zones of interest. We also present the results of a case study on the co-occurrence between a corn herbicide and fifteen federally listed species.

05.01.04 Aquatic Exposure Refinement for Endangered Species Assessments Considering Multiple Use Patterns Across Different Scales

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Within any endangered species risk assessment framework, quantitative pesticide exposure and risk modeling is a powerful tool to make the important distinction between threatened and endangered species that are likely to be or not likely to be adversely affected by the action (registration of a pesticide). A multi-tiered approach for national scale aquatic exposure modeling was developed, where the types of waterbodies evaluated range from small ponds with a single field fed by a 10 ha watershed to very large flowing waterbody systems with watersheds greater than 10,000 km^2. The process begins with a highly conservative, Tier 1, approach, based upon standard USEPA models and assumptions. At Tier 2, spatial crop and usage data are incorporated into the exposure modeling in three refinement steps. The first refinement accounts for potential pesticide use sites, or percent cropped area (PCA). The second refinement step is based on best available pesticide usage data resulting in multiple realizations of use footprints that are incorporated into the exposure models as percent crop treated (PCT) values. In both of these refinement steps, watershed-specific estimated environmental concentrations (EECs) are calculated by summing the PCA or PCT adjusted 1-in-15-year annual maximum use pattern-specific concentrations to determine the multi-use pattern aggregated EECs for the watershed and waterbody. While this approach is efficient because no new model runs have to be conducted, it conservatively assumes that the peak events from different use pattern and crops are occurring at the same time (maxima are added even if they are not coincident in time). Thus, the third refinement step of Tier 2 accounts for the differences in application timing between use patterns by first creating a PCA/PCT-adjusted time series of the loadings entering the waterbody originating from multiple use patterns throughout the watershed and then using it as input for the receiving water model. This approach is congruent with the highest tier assessment level proposed by EPA when integrating PCA and PCT into drinking water assessments. This presentation will provide an overview of the impact of this refinement step. The results show that this step can be a significant refinement but is dependent upon the number of different use pattern present in the watershed, often a function of the watershed size.

05.01.05 Integrating Landscape-Level Agronomic Information Into Aquatic Exposure Estimates: Approaches and Benefits

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Prospective aquatic exposure modeling is a key aspect in the assessment of potential jeopardy of endangered species during the preparation of a Biological Opinion (BO) related to the federal action of pesticide (re)registration. The Biological Evaluation (BE) prepared by the EPA provides the foundation of aquatic exposure estimates using well-established pesticide fate and transport models (e.g., PWC, PRZM, VVWM, AgDRIFT) applied to a standardized set of crop/soil/weather scenarios. Exposure estimates are based on 30 years of model simulation implementing labeled uses of the pesticide specifying maximum application rates, number of applications, re-application intervals and assuming treated crop is adjacent to the aquatic environment. This screening-level approach forms the basis from which subsequent refinements incorporating spatial, temporal, and pesticide usage variability can be applied to better inform the weight of evidence process. This presentation will describe the purpose, design decisions, assumptions and uncertainties, along with results of these post-screening refinements as related to the recent carbaryl BE. Variability in such aspects 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 as well as agency guidance documents. The implementation of these refinements shows that the 1-in15 year EECs based on screening level assumptions of pesticide use and hypothetical water body scenarios may occur at some locations at limited times, but they are far less likely to occur (within species range/habitat) than is assumed in screening-level risk assessments. The methodologies applied within these refinements allow for a quantitative approach for incorporating variations in landscape and agronomic practices near endangered species locations.

05.01.06 Integrating Landscape-Level Agronomic Information Into Aquatic Exposure Estimates: Proximity and Cropping Density

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The spatial relationship between potential pesticide use sites and Endangered Species locations (e.g., range, critical habitat, suitable habitat, known locations) determines exposure and is an influential factor in evaluating potential risk to these species. We developed models and processing approaches to characterize spatial relationships in an efficient and documented manner. As a first step, we characterized potential pesticide use sites (i.e., Use Data Layers, UDLs) in relation to species aquatic habitats. As a second step, we defined the spatial extent of species habitats using the NHD+ framework of flowlines, catchments and static water bodies which formed the basis for our spatial definition of habitat. Each water catchment was characterized in terms of total UDL area forming the basis for Percent Crop Area (PCA) refinements applied to baseline PWC Estimated Environmental Concentrations (EECs) concentrations. Further, eight proximity zones (PZs) around the water bodies were spatially defined in order to quantify UDL areas within each PZ. The use of proximity zones is significant for refinement of ESA assessments as PZ cropping density can incorporate relevant landscape-based drift deposition and runoff/erosion information. We also quantified, for each catchment and UDL, the proportion of crop within the catchment that was greater than 300m (~1000') or 900m (~2600') beyond which spray drift deposition no longer contributes to the water body EEC (from ground and aerial applications, respectively). The ability to characterize variability in potential exposure within and between catchments enabled a quantitative and visual distribution of potential exposure across a species range or critical habitat. This landscape-specific information supports spatially-informed, species-specific exposure analyses as an essential line of evidence for the Biological Opinion. This presentation will describe the design and practical implementation of spatial proximity and cropping density approaches for listed species.

05.01.07 Integrating Landscape-Level Agronomic Information into Aquatic Exposure Estimates: Efficiency and Reproducibility

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The challenge of evaluating exposure potential from multiple labeled uses for over 1600 Endangered Species nationally can be intimidating. It is important to be as spatially detailed as possible in order to identify potential product use areas and regional factors that are most relevant to each species being evaluated. In addition, with over 1600 species being evaluated for a multitude of labeled uses modeled on 13 agricultural Use Data Layers (UDLs), efficiency and reproducibility are critical. We developed a highly efficient and structured approach that incorporates best available information in order to increase the reliability and relevance of exposure estimates to listed species. The refinement framework incorporates additional dimensions of aquatic exposure modeling such as cropping density and proximity, pesticide treated acre distribution, and consideration of temporal exposure. A structured and programmatic framework was implemented to build upon the baseline scenario concentrations, and apply spatial, temporal and agronomic aspects to produce well-defined and reproducible species-specific aquatic concentrations. In this presentation we will describe how daily data from thousands of 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. Utilizing those data for each species, the set of NHD+ catchments, landscape-based crop proximity and density, pesticide usage (e.g., Percent

Crop Treated), and user options (e.g., PCT multiplier, alternate application rates) were processed to produce refined exposure concentrations suitable for aggregation at multiple spatial scales appropriate for the species being examined. Transparency of input data, modeling run options and availability of raw and formatted results was paramount in this process and resulted in the ability to rapidly process and document alternative cropped areas, application rates, intervals and other variables.

05.01.08 Developing Refined Pesticide Risk Assessment Models for Endangered Species: A Case Study for the Cape Sable Seaside Sparrow

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The U.S. Environmental Protection Agency (EPA) uses a conservative, screening-level approach for national endangered species Biological Evaluations. This has led to a high proportion of listed species being deemed likely to be adversely affected (LAA) for pesticides evaluated to date. As a result, the U.S. Services must generate Biological Opinions involving hundreds of listed species per pesticide. Refined species-specific models are needed to determine if risk to each species or their critical habitat is reasonably certain. The Cape Sable Seaside Sparrow (CSSS) is only found in mixed marl prairie and marshy habitats in South Florida. A screening-level risk assessment using EPA's conservative approach did not screen out the CSSS for potential risk from exposure to a representative insecticide. Thus, a probabilistic, spatially-explicit exposure and risk model was developed to assess the risks of pesticides to the CSSS during the breeding season. The model simulates acute exposure to 1,000 birds and their invertebrate prey over a 60-day period following initial application. The model time step is ten minutes, approximating the interval for foraging trips from the nest. At each time step, the model randomly determines distance between bird foraging location and the closest edge of the closest treated area. A spray drift model determines fraction of applied insecticide reaching the foraging location, which determines concentrations in terrestrial invertebrates. For aquatic invertebrates near the foraging location, a simple partitioning model is used to estimate concentrations in these organisms. CSSS body burden is determined by combining total intake (diet and drinking water) at each time step with the rate of elimination for the birds. Direct acute risk is estimated by comparing the peak body burden for each simulated bird to a randomly chosen value from the dose-response curve for a sensitive test species. If the peak body burden exceeds the randomly chosen effects value, the bird is assumed to be dead. Otherwise, the bird survived. This procedure is repeated for 1,000 simulated birds for each crop group. For aquatic and terrestrial invertebrate prey items, the model estimates probabilities of exceeding the corresponding most sensitive NOECs. In the presentation, we will illustrate model application with a representative insecticide as well as consider other lines of evidence.

05.01.09 Integration of Landscape Scale Information to Reduce Uncertainty in Endangered Species Assessments and Protection Strategies for Pesticides

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The protection of federally listed threatened and endangered species requires a multidisciplinary approach that leverages best available data to accurately identify and address risk to species and their habitats. Assessment and management uncertainty can be significantly reduced by applying localized spatial and temporal landscape information along with information about potential stressors. Several pieces of information should be collected at the onset of each assessment to address potential risk including 1) an understanding of where the species and its habitats are most likely to occur in the landscape, 2) identification of factors or conditions required to support the species, 3) knowledge and prioritization of stressors already identified as influential to species status, 4) a determination of where the stressor being evaluated is likely to occur on the landscape relative to species and its habitats, 5) an assessment of the potential degree to which the stressor may influence the species. This information can be used for targeted management strategies regarding the unique relationship between the species and stressors being evaluated. State and federal agencies often collect extensive information on species status, requirements, habitats, influencing stressors and recovery goals which can be used to develop the information set described above. In cases where pesticides are being evaluated as a potential stressor, registrants often have extensive product knowledge that can be applied to further reduce uncertainty in these assessments. We present an approach using a model insecticide that integrates species and product information at a local, species-centric scale. Several examples are presented that outline a pragmatic approach to reducing assessment uncertainty so that species management strategies and resources can be effectively applied. These general approaches can be used for many pesticides and will facilitate a more efficient process for evaluating risk that may, or may not, be reasonably certain to occur for species.

05.01.10 Alternative Approach to Conducting Step 2 of an Herbicide Biological Evaluation

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The US Environmental Protection Agency (EPA) developed a Revised Method to conduct pesticide biological evaluations (BE) for species listed under the Endangered Species Act. Step 1 examines where the listed species are located, and pesticides applied to determine whether there is potential for exposure. An evaluation of whether there is potential for adverse effects using screening-level tools is then conducted. Step 2 slightly refines this analysis but due to compounding conservatism, fails to distinguish listed species that are reasonably certain to be at risk from those that are unlikely to be. An alternative approach for conducting Step 2 is described herein. It contains several elements common to the EPA herbicide BEs, including consideration of usage data, use of the new Plant Assessment Tool, and probabilistic methods. However, this alternative Step 2 analysis considers these elements differently. The goal is to quantify potential risk to individuals of each species within their ranges that is reasonably certain to occur. The key elements of this approach are: · Exposure: The terrestrial exposure modeling approach integrates usage analysis and percent crop treated (PCT), proximity of listed species to use sites, and exposure models to quantify both the likelihood and magnitude of exposure. · Effects: A method to generate effect metrics that are unbiased has been developed. For example, a species sensitivity distribution (SSD) can be derived using the standard crop test species. · Risk Assessment: For each of 10,000 simulated individual plants, direct risk can be expressed as percent inhibition of growth by determining where estimated exposure occurs on the exposure-response curve. Risk curves are generated for each species. · Risk Characterization: All lines of evidence (e.g., field studies, life history information, incident reports) enter the overall weight of evidence assessment for each listed species. The effect determination is based on all evidence. The Step 2 alternative methods demonstrate that risk of adverse effect can be quantitatively characterized and evaluated to determine whether they are reasonably certain to occur. The methods follow the concepts of probabilistic modeling for exposure, effects, and risk (advocated by the National Academy of Sciences), and account for the best available data (e.g., usage) in an efficient and pragmatic way when estimating magnitude and likelihood of exposure.

05.01.11 A Probabilistic National Endangered Species Exposure Assessment for Terrestrial Plants Using the Plant Assessment Tool (PAT)

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Quantitative pesticide exposure and risk modeling is a powerful tool to make the important distinction between threatened and endangered species that are reasonably likely and not likely to be adversely affected (LAA and NLAA, respectively) as a result of a pesticide's labeled uses. Terrestrial species found off-field may be exposed via off-target spray drift or runoff. For both exposure pathways, understanding the proximity of pesticide use sites to endangered species locations is an important component of evaluating potential risk. National land use datasets provide the foundation for spatial analysis methods that help quantify species range proximity to potential pesticide use locations. Multiple years of land use and cropping data can account for temporal variability in cropping patterns and uncertainty in crop classification, both of which are necessary to accurately characterize potential exposure for a species. A proximity analysis can be structured to produce probability distributions of proximity distances for an entire species range, providing robust datasets suitable for use in exposure models. Typically, a proximity analysis assumes that 100% of potential use sites receive pesticide applications. Although an acceptable conservative assumption in a screening-level assessment, this assumption does not reflect reality based on best available data. Pesticide usage often occurs on only a fraction of labeled potential use sites. Therefore, refinement of the use locations is important to better quantify proximities. We have developed a probabilistic methodology for modeling terrestrial exposure using the Plant Assessment Tool (PAT). The PAT is a mechanistic model that estimates pesticide concentrations in terrestrial, wetland, and aquatic plant habitats and was recently released by the US Environmental Protection Agency (EPA). Our national scale modeling approach incorporates multiple realizations of pesticide usage footprints and the outcome of a proximity analysis to quantify the likelihood of pesticide usage occurring within a distribution of proximity distances throughout species ranges. The results of this analysis are species-specific probability distributions of exposure exceedances that can be incorporated directly into risk models for terrestrial species and included as part of a weight of evidence assessments.

05.01.12 A Stepwise Approach to Refining Risk Assessments for Listed Terrestrial Plants

M.E. Kern, Balance EcoSolutions; N. Snyder, N. Guth, Waterborne Environmental, Inc.; J.M. Jackson, Waterborne Environmental, Inc. / Effects Assessment; T.M. Blickley, Corteva agriscience / Ecotoxicology; P. Havens, Corteva agriscience

The refinement of ecological risk assessments for listed species continues to evolve as methods are developed and best available scientific data and information are introduced. National scale assessments conducted by the EPA consider the potential exposure to at least one individual of a listed species or its supporting habitat (e.g., plants, pollinators, prey). Conclusions from these assessments are primarily made based on the assumption that users will apply the product on all potential use sites using maximum labeled application options (maximum rates and number of applications, shortest application interval, application methods that produce the greatest drift, etc.). Assessments conducted by the US Fish and Wildlife Service and National Marine Fisheries Service (Services) consider these findings to determine if impacts are reasonably likely to occur for the species population and supporting habitats. The Services may consider additional species information and typical product use and usage information. Addressing uncertainties identified in the early phases of the ecological assessment can sometimes be limited by resources instead of data availability when an analysis plan is not well established. Assessment conclusions can be significantly limited without the application of existing best available scientific data and information such as product usage, typical application methods, spatial detail, and species information to address these uncertainties. This presentation illustrates a stepwise approach demonstrating the efficient application of such information as part of a refined assessment for listed plants. The assessment addresses both direct effects to plants and potential effects to supporting pollinators using a representative insecticide. Multiple lines of evidence are applied to determine if specific product use scenarios are likely to result in exposures to specific listed plants and pollinators. The assessment highlights the importance of incorporating the most relevant and reliable information to reduce uncertainty and improve our understanding of potential pesticide risk to listed species.

05.01.13 The Application of Species Attributes in Listed Terrestrial Plants Refining Risk Assessments

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The incorporation of best available species information in ecological risk assessments for listed species offers a significant opportunity to advance risk characterization and reduce uncertainty in these assessments. The US Fish and Wildlife Service, National Marine Fisheries Service, and other state and conservation organizations often provide extensive species biological data that can then be used to support these efforts. For example, localized species protection and recovery goals from management plans typically provide detail on sensitive areas where management is most influential (e.g., critical spawning areas or species known locations). Many of these plans contain detailed information on the species (e.g., habitat, reproduction, and distribution) baseline status of the species, and its stressors (e.g., urban development, catastrophic weather events, and invasive species) that most likely influence the status and potential recovery of the species. Details on interspecies relationships (e.g., dietary choices and pollinators), help to understand relevant routes of exposure and can be used to inform how toxicity of the compound being evaluated is applied to the assessment. All this information clarifies the relationship between a stressor and the species being assessed. This presentation focuses on the application of these data to advance a refined ecological risk assessment for terrestrial plant species as part of a stepwise approach. Species attributes are applied to reduce assessment uncertainty and characterize potential risk to enable more effective species management strategies.

05.01.14 Refining Spatial Analysis Using Best Available Species Attributes for Listed Terrestrial Plant Species

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The incorporation of best available species information in ecological risk assessments for listed species offers a significant opportunity to refine spatial analysis to reduce assessment uncertainty. The US Fish and Wildlife Service provides range and critical habitat spatial data for listed plant species which can be combined with USDA cropping and compound usage data to inform overlap and impact of usage on a risk assessment. The spatial-specific cropping and usage data are often collected and developed over time and are available at variable specificity for different species. The species biological data, 5-year review and recovery plan documents produced by the US Fish and Wildlife Services, National Marine Fisheries Service and other state or conservation organizations often provide detail on sensitive areas where management is most influential (e.g., critical spawning areas or species known locations). Most notably, species specific habitat requirements are very useful to inform identification of suitable habitat, potential utilization of various land cover types, and relationships to potential product use sites. This narrative information, combined with spatial data provided by the USGS, USDA, and state or local agencies (such as elevation, land cover, soils, and park/administrative boundaries) can aid in the identification of species suitable habitat at often quite detailed spatial resolution. Such spatial identification of a species suitable habitat and potential areas of product use can clarify the relationship between a stressor and the species being assessed and thus refine the risk characterization for the species. This presentation focuses on the application of these methods to visualize the spatial relationship between areas most suitable for a species and areas of potential product use for a representative insecticide.

05.01.15 Pesticide Analysis of Composited Hourly Surface Water Samples From an Orchard Dominated Watershed

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Since 2003, the Washington State Department of Agriculture (WSDA) has been charged with assessing the frequency and biological significance of pesticides in surface waters and providing voluntary best management practices for reducing off-target movement. WSDA has been collecting weekly grab samples across the state, including Brender Creek in Chelan County, since 2007. Beginning in 2018, grab samples revealed simultaneous detections of chlorpyrifos and malathion at concentrations above EPA's Aquatic Life Benchmarks. Grab sample results represent a snapshot in time. Laboratory toxicity studies often occur over days to weeks and EPA's Biological Evaluation modeling produces 1 in 15 year daily average estimated environmental concentrations. This time scale discrepancy creates uncertainty in the ability to assess risk of off-target pesticides in aquatic settings. To reduce uncertainty, and due to the elevated detections of malathion and chlorpyrifos, WSDA designed a project to better assess exposure and provide "best available" data through composite sampling. Autosamplers collected water hourly and composited for 12 to 24 hour periods from March 30th to April 17th 2021 (19 days), coinciding with expected peak spring pesticide applications. Three weekly grab samples were collected for comparison to composite samples. Twenty four hour average concentrations ranged from 0.114 to 0.0117 and 0.409 to 0.00558 µg/L for chlorpyrifos and malathion, respectively. When considered individually, chlorpyrifos and malathion concentrations in the composited samples were above the acute endangered species level of concern (ESLOC) for the first 5 and 3 consecutive days, respectively. The ESLOC sum of toxic units for these insecticides was >= 1.0 for 9 of the first 10 days. One grab sample malathion result was five times the corresponding 24 hour composite sample. These data suggest that an unacceptable level of risk to endangered species and their habitat occurred during the study period. Application timing, practices, daily density of applications and weather in the watershed could all have an impact on the differences in concentrations seen throughout the duration of the study period. New application timing tools are in development and further education activities are planned for the watershed in the future, targeting reduction of off-target movement in early spring applications through voluntary best management practices.

05.01.16 Conservation, Pesticides, and Endangered Species

A.R. Frank, Compliance Services International (CSI); L.M. Duzy, Compliance Services International; T.B. Fredricks, Bayer CropScience / Global Regulatory Sciences; J. Marton, Corteva agriscience; T. Burd, Syngenta Crop Protection LLC

American farmers and ranchers play a key role in the stewardship of their land and surrounding ecosystems on which we all rely. A productive agricultural system includes pest and nutrient management techniques, many of which can be conservation practices, such as edge-of-field filter strips, that help improve and protect the soil, air, and water quality. These conservation practices can also limit the inadvertent and undesirable movement of pesticides beyond the application area thereby reducing potential pesticide exposure of surrounding habitats. The Federal Insecticide, Fungicide, Rodenticide Act Endangered Species Task Force (FESTF) is exploring how various conservation practices can serve to prevent or reduce pesticide exposure to threatened or endangered species and their designated critical habitat. The goal of the efforts being explored by FESTF is to identify options for how new and existing conservation practices and measures can be both achievable and realized within the framework of pesticide risk assessment and the Endangered Species Act. This presentation will provide an overview of FESTF's efforts, detail a pilot program, and discuss next steps.

05.01.17 Species-Specific Avoidance in Endangered Species Act (ESA) Assessments of Pesticides: A Proposed Strategy for Determining Appropriate Measures and How to Implement Them

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The process to assess the potential of a pesticide to jeopardize endangered species continues to evolve with input from regulatory agencies, industry, non-governmental organizations, and other stake holders. The current approach does not consider possible measures (or "avoidance") to help address potential jeopardy until the very end of the process when the Services might issue Reasonable and Prudent Alternatives (RPAs) intended to reduce exposure and/or Reasonable and Prudent Measures (RPMs) intended to minimize "incidental take". However, proposed avoidance measures could be integrated earlier in the assessment process to develop targeted, species-specific solutions that address potential jeopardy concerns while avoiding unnecessary impacts to growers. This presentation will describe a proposed strategy for identifying the potential need for avoidance in a targeted manner, possible ways to incorporate that information into the ESA assessment as early in the process as possible, and how the avoidance can be implemented.

05.01.19 AMMPS: Informing Discussions on Avoidance, Minimization, and Mitigation for Pesticides and Species

L.M. Duzy, A.R. Frank, Compliance Services International (CSI); T. Burd, L. Ghebremichael, Syngenta Crop Protection, LLC; Y. Li, Environmental Policy Innovation Center; D. Campbell, Independent Consultant

Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the registration of a pesticide requires compliance with the Endangered Species Act (ESA) which includes evaluating the potential risk of the use of the pesticide on ESA-listed species and designated critical habitats. For the agencies responsible for registration and consultation, this can be an extensive exercise involving many complex datasets and processes, as demonstrated in recent draft biological evaluations and draft biological opinions from the US Environmental Protection Agency (EPA), the US Fish and Wildlife Service, and the National Marine Fisheries Service (collectively, the Services). For the pesticide registrant seeking registration, this can present an opportunity to explore ways to adequately protect ESA-listed species while also considering what is feasible for the end-user. We developed a tool that indexes factors that are important to the analysis of risk, as well as imperilment and conservation needs of each ESA-listed species. With these factors, AMMPS, a tool for informing discussions on avoidance, minimization, and mitigation for pesticides and species, sequences species so that the registrant, EPA, and the Services can have informed discussions about conservation measures that may be the most appropriate for each species in the FIFRA/ESA assessment and consultation process. The objective of this presentation is to demonstrate AMMPS using an herbicide used on cultivated land in the contiguous United States as a case study.

05.01.20 A Pilot Example of Maximizing Conservation Measures: Using Avoidance, Minimization, and Offsetting in National Scale Pesticide Consultations

J. Bickel, Creekbank Associates; D. Perkins, D&E Technical, Inc.; W. White, W-Squared Consulting; M. Thabault, Creekbank Associates; S.R. Mortensen, BASF Corporation

Evaluation of effects for listed species in national-scale pesticide registrations inherently holds a great deal of uncertainty, however uncertainty and data variabilities should not preclude the use of all available conservation measures. In this pilot, we developed effects assessment methodologies that were used to quantify residual, unavoidable impacts following avoidance and minimization measures. These advancements in Endangered Species Act (ESA) effects analysis can improve the project description in ESA section 7 consultation(s), including ESA-explicit label mitigations associated with a pesticide registration action. Currently under jeopardy circumstances, reasonable and prudent measures (RPMs) and reasonable and prudent alternatives (RPAs) have been the primary mechanism utilized by the consulting agencies for pesticide project actions. However, the full suite of conservation measures (avoidance, minimization, and offsetting) have not been applied. During informal consultation, all forms of conservation measures can be leveraged to result in non-jeopardy determinations. In this way, conservation offsets provided by the registrant account for these remaining residual, unavoidable impacts to listed species. From this pilot project, we concluded that the conceptual effects framework we developed: 1) quantified residual, unavoidable impact, 2) offered greater flexibility in business decision-making associated with market reach and conservation commitments, 3) provided defensible outcomes toward ESA compliance that can be demonstrated, 4) identified and prioritized conservation opportunities, and 5) could be scalable for use within grouped registration actions, for multiple species, and for the implementation of agreed-upon offset measures. We also report on the collaboration process between FWS, EPA, and State conservation agencies. The need for meaningful conservation outcomes and an effective consultation process for pesticide registration remains a challenge; however, this pilot demonstrates that use of crop protection products is not at odds with environmental stewardship and regulatory compliance.

05.01.21 Developing a Communication Platform that Meets the Needs of Both FIFRA Labeling Instructions and Endangered Species Act Avoidance and Minimization Requirements

D. Perkins, D&E Technical, Inc.; J. Bickel, Creekbank Associates; M. Von Hendy, Green Heron Information Services; W. White, W-Squared Consulting; M. Thabault, Creekbank Associates; S.R. Mortensen, BASF Corporation

There are an increasing number of pesticides that are moving through the Federal Environmental Protection Agency (EPA) endangered species biological evaluation toward formal Endangered Species Act (ESA) Section 7 consultation. One of the principal outcomes of the Section 7 consultation and EPA registration review process is the end-use product label that includes application instructions. Pesticide labels may include 'label mitigations,' which are legally binding application instructions related to environmental and human health risk specifically identified through the EPA registration review process. As pesticides complete the ESA Section 7 consultation process for endangered species, a new set of label instructions will likely be needed to adequately protect listed species. Currently, EPA uses Bulletins Live Two! as the primary mechanism to communicate label instructions to growers and professional pesticide applicators related to endangered species; however, a revised technological platform with an expanded scope is needed to effectively communicate both Federal Fungicide Insecticide and Rodenticide Act (FIFRA) and ESA-related application instructions. In this work, we discuss critical elements and digital platforms that would better meet the needs of both FIFRA registration and ESA Section 7 label instructions, including: 1) spatial resolution, 2) temporal resolution, 3) proximity to sensitive habitat, 4) application technologies, 5) environmental conditions constraints, and 6) possible application reporting features. In addition to the technological aspects of a revised label instruction and communication platform, specific listed species mitigations resulting from both FIFRA registration and ESA Section 7 consultations can be dynamically developed during the consultation process as agencies work together to find solutions that work for growers and listed species alike. Such a revised platform should also allow for integration of best-available scientific data and information while simultaneously providing near field-scale label instructions and protecting explicit locations of extant listed populations, as useful for label instructions and compliance. Finally, recent advances in digital technology allows for a more fluid user experience that can be a non-trivial improvement toward providing useful instructions as well as compliance.

05.02 Background: Soil, Sediment, Groundwater, Surface Water and Air – Cleanup Site Risk Assessment and Remedial Decision Making

05.02.01 Using the Region 4 Urban Background Study to Establish Site-Specific Background in Chattanooga, Tennessee

S. Chan, Environmental Protection Agency

Historically, over sixty (60) foundries operated in and around the City of Chattanooga, Tennessee. Waste material from the foundries was available to residents for use as fill material. Some of the foundry waste used as fill material contained hazardous levels of lead. An urban background study was conducted in the City of Chattanooga to understand if elevated lead concentrations were evidence of a "CERCLA release" or if the elevated lead levels found in residential yards could be attributed to anthropogenic background. Using a modified sampling and analysis plan for prior work conducted under the EPA Region 4 Urban Background Study, a 5-mile by 5-mile grid was laid over the city core and discrete samples were obtained from 50 randomly-selected grids and analyzed to establish urban background concentrations of metals and PAHs. Analysis of background soil samples showed that background concentrations of lead were statistically different from the concentrations found in contaminated residential soils, confirming that residential soils were above anthropogenic background. The collection of a robust anthropogenic background dataset established site-specific background for metals and PAHs and helped to inform the site-specific clean-up goals used at the Site.

05.02.02 Upcoming ITRC Soil Background and Risk Assessment Guidance and Training Videos

B. Brooks, Washington Department of Ecology / Toxics Cleanup Program; C. Sorrentino, California Department of Toxic Substances Control; L.H. Wilson, J. Rocco, Sage Risk Solutions, LLC

Risk assessors use soil risk-based screening levels (RBSLs) for chemicals commonly found in soil to evaluate whether they could potentially pose risks to people or the environment. For some chemicals, the RBSLs are well within their natural or anthropogenic ambient background concentrations. For example, arsenic is frequently found naturally in some soil at concentrations higher than its RBSLs. Some organic chemicals, such as dioxins, may be found in soil at concentrations higher than RBSLs from anthropogenic ambient sources not related to site releases. Consequently, considering natural and anthropogenic ambient background is important for risk assessment and to inform risk management decisions. Although some states, federal agencies, and other entities have guidance documents regarding this subject, there is no comprehensive up-to-date guidance or summary of existing resources that is widely applicable. The Interstate Technology and Regulatory Council (ITRC) Soil Background and Risk (SBR) guidance and training videos will provide a framework for establishing soil background and using it in risk assessment. The guidance will be in the form of a web-based document available on the ITRC website in December 2021. Topics covered by the training videos are expected to include natural and anthropogenic ambient background, sampling and analytical methods pertaining to soil background, establishing default and site-specific soil background, using default and site-specific soil background, and geochemical evaluations and environmental forensics relevant to determining soil background. The training videos will also be accessible online for on-demand viewing. Online training events offered on the U.S. EPA CLU-IN platform will incorporate presentations and videos addressing the main topics followed by a live question and answer session.

05.02.03 Soil Background Evaluations in Risk Assessment: Current Regulatory Frameworks

K. Patel-Coleman, Burns & McDonnell; J. Dyson, GHD; B. Brooks, Washington Department of Ecology / Toxics Cleanup Program; C. Sorrentino, California Department of Toxic Substances Control

Site management decisions at chemically impacted properties are usually based on human and/or ecological risks. However, sometimes risk-based, protective concentrations for some chemicals in soil can be lower than concentrations present in the environment from natural sources or anthropogenic ambient background. In these cases, some regulatory agencies typically would not require responsible parties to clean up soils to risk-based levels but rather to background concentrations. Thus, evaluating soil background levels at a site and its implications for risk assessment can make a significant difference in overall clean-up time and costs. A limitation in realizing adequate soil background chemical evaluations, especially as they apply to risk assessments, is that approaches vary widely across states. Some states have no guidance, others provide limited information, and many that do have guidance do not incorporate advanced statistical, geochemical or forensic methods that could richly inform the background evaluation. For this reason, the Interstate Technology and Regulatory Council (ITRC), a state-led-coalition of regulators, private sector professionals, academia, the public and tribal stakeholders, launched a team in 2020 to develop national guidance on conducting soil background studies for risk assessment. This guidance will be published in December 2021 and will be available on ITRC's website. Development of the ITRC guidance document included surveying state agencies and researching existing state guidance and policies on soil background evaluations and their use in risk assessment. In this presentation, we will summarize the results of this research and present the current regulatory frameworks surrounding soil background in risk assessment.

05.02.04 The Importance of Evaluating Ambient Conditions in Risk Assessments Performed as Part of a Multi-Site Program

M.W. Kierski, Exponent / Ecological and Biological Services Practice; C. Menzie, Exponent / Ecological & Biological Sciences; K. O'Reilly, Exponent, Inc.; S. Meyer, Ramboll; P. Kenny, WEC Energy Group – Business Services

With a long history of industrialization, and a historic city-wide fire, the City of Chicago has received pollutant loading for centuries from a number of different sources. Identifying the input of a common environmental pollutant, polycyclic aromatic hydrocarbons (PAHs), from a specific source - former manufactured gas plant (MGP) sites - is complicated by current and historical PAH contributions from a myriad of sources over this timespan. For each MGP site, a baseline risk assessment is performed to address the potential risks associated with legacy PAH contamination associated with the former MGP operations that ceased decades ago. Characterizing the ambient PAH conditions in air, soil, surface water and sediment has been an integral part of the risk assessments performed to help identify site conditions that depart from ambient conditions and require remedial action. The ambient conditions in air, soil, surface water and sediment have been characterized at a number of the MGP sites located along the Chicago River as part of a Multi-Site MGP Program. Ambient air concentrations have been evaluated and factored into the risk assessments performed to identify contamination needing remedial actions to protect indoor air quality. The ambient PAH conditions in the City of Chicago soils were factored into an approach used to define limits of MGP related PAHs in the upland areas of these sites and characterize the risk of soil exposure. In addition, an ambient surface water and sediment investigation and evaluation approach, which was first used on the North Branch of the Chicago River, was expanded to other reaches of the Chicago Area Waterway System to identify the ambient PAH conditions spatially and the associated risks. The risks identified at these MGP sites associated with human or ecological receptor exposures to each media (air, soil, surface water and sediment) were characterized and compared and contrasted to the risks associated with ambient conditions. The importance of evaluating ambient conditions as part of a risk assessment in each medium will be presented as a series of case studies. An important lesson learned is that ambient conditions in some urban areas themselves are associated with risk levels that are above regulatory limits. From an environmental justice perspective, this points to the fact that there is a broader social impact of these urban ambient conditions that needs to be addressed.

05.02.05 Geochemical Evaluations of Metals and the Determination of Background

K. Thorbjornsen, APTIM

Metals detected in soil, sediment, groundwater, and surface water samples often exceed risk-based screening levels in the absence of site-related or anthropogenic impacts. This is true of trace elements such as arsenic and copper, as well as major elements such as aluminum and iron. Distinguishing naturally elevated concentrations from contamination is therefore critical during the development of background data sets and subsequent comparisons of site versus background data sets. Statistical approaches can help somewhat, but they are unable to provide mechanistic explanations for the geochemical processes controlling element concentrations. This is where a properly performed geochemical evaluation provides great value, by not only pinpointing individual samples that have contaminant impacts but also revealing the relevant geochemical processes that control natural background concentrations at a site.Geochemical evaluations are based on well-established associations between trace elements and specific minerals in soil and sediment (or suspended minerals in groundwater and surface water); and they consider site-specific processes such as adsorption-desorption reactions, redox effects, pH effects, evaporative concentration, and the presence of mineralized zones. Trace-versus-major element associations are visualized with scatter plots and ratio plots (among other exploratory data techniques). Samples with anomalously high elemental ratios may indicate the presence of contaminant input and are examined further using all available information and analytical data.Representative case studies are presented from background studies and site-to-background comparisons for metals in soil, sediment, and groundwater at a variety of sites. These geochemical evaluations have been successfully applied at mine sites, petroleum refineries, munitions sites, industrial/chemical sites, and military installations across the U.S. The results have been used during site investigations to refine lists of chemicals of concern, optimize long-term monitoring programs, and verify the success of cleanup efforts. During background studies, geochemical evaluations have been used to determine whether statistical outliers represent background concentrations and whether subgroups of data (e.g., samples from different depths) can be combined. Attendees will find immediate applicability to their own sites.

05.02.06 Incremental Sampling Methodology, Growing into an Everyday Tool

M. Bruce, Eurofins | Environment Testing; H. Brittingham, Neptune and Company, Inc.; M. Zych, Wood Environment & Infrastructure Solutions Inc

In October 2020, the Interstate Technology and Regulatory Council (ITRC) released an updated incremental sampling methodology (ISM) guidance to reflect advancements in technology and share case studies providing insight into the application, benefit and pitfalls of ISM. This talk will introduce the main concepts and provide direction for implementation. There is more information on soil heterogeneity and interactive examples for systematic planning. A cost section provides site specific financial analysis guidance. Field implementation and sample processing methods are updated and new tools are described. Data uses include site characterization, confirmation sampling, and risk assessment purposes. The ITRC Soil Background & Risk guidance also includes the use of ISM.

05.02.07 Balancing a Background of Bioaccumulatives and Protectiveness When Managing Sediment in Washington State

C. Asher, Washington State Department of Ecology / Toxics Cleanup Program; L.S. Inouye, Washington State Department of Ecology / Shorelands and Environmental Assessment

In 1991 Washington state adopted the Sediment Management Standards regulation, providing a regulatory structure for managing contaminated sediment, dredged material, and controlling sources—the first U.S. state to adopt this type of regulation. At the time of adoption, the regulation included marine benthic criteria and narrative standards for protection of human health and the freshwater benthic community. In the proceeding decades the science surrounding the toxicology and fate and transport of bioaccumulative chemicals in the aquatic environment evolved. Due to the high fish and shellfish consumption rates in Washington state and the diversity of aquatic wildlife—including a number of threatened and endangered species—risk assessments can result in risk-based criteria for bioaccumulative chemicals well below the benthic criteria, sediment background, and analytical quantitation limits. These low risk-based criteria, along with the ubiquitous nature of bioaccumulative chemicals, would make cleanup standards infeasible to attain and maintain and site boundaries very hard to establish—making cleanup impracticable. In 2013 the state updated the Sediment Management Standards to include standards for bioaccumulative chemicals based on ecological and human health risk, analytical quantitation limits, and background. The regulation includes two levels of background: 1) natural background, which includes naturally occurring and anthropogenic chemicals from global sources, and 2) regional background, which includes chemicals from diffuse urban sources that are not primarily attributable to identifiable sources. These background-based standards can be used for cleanup if higher than risk-based levels and quantitation limits. This allows some finality for cleanup sites despite the potential of recontamination from ongoing ubiquitous sources, which may require decades to reduce or control. The presentation will include the challenges and lessons learned—particularly in defining sediment background in both marine and freshwater environments.

05.02.09 State of Washington Natural Background Groundwater Arsenic Levels

C. San Juan, WA Dept of Ecology

Frequent detection of low levels of arsenic in groundwater (e.g. 5-15 ug/L) at Washington Department of Ecology cleanup sites (hazardous waste, etc.), is a common occurrence. In many cases, these low levels are the result of naturally occurring conditions (e.g. reduced groundwater from organic rich soil, etc.). However, degradation of groundwater petroleum (or other volatile organics) can result in low levels of arsenic as well. Ecology's current groundwater arsenic cleanup level based on protection of people and the environment is 5 ug/L. In some areas of Washington, this cleanup level is lower than arsenic natural background levels in groundwater. Since you cannot cleanup groundwater to levels that are less than natural arsenic background, this has presented a challenge when setting cleanup site remedial goals. This presentation provides details on a study that was done to update Ecology's groundwater arsenic level. The most important finding of this study is that Ecology's current groundwater arsenic standard of 5 µg/L is at the low end of the statewide natural background range (4.9 – 14.6 µg/L). Thus, Ecology plans to revise the current cleanup level of 5 ug/L to values from this study.

05.02.10 Rapid Assessment of Background Indoor Air Concentrations and Reasonable Maximum Exposures from Vapor Intrusion of Volatile Organic Compounds

H. Dawson, T. Gabris, Geosyntec Consultants, Inc.

The impact of vapor intrusion (VI) on indoor air is challenging to assess using conventional monitoring approaches because of temporal variability in volatile organic compound (VOC) concentrations arising from VI and off-gassing of VOCs from background sources. These challenges lead to uncertainty in identifying a Reasonable Maximum Exposure (RME) for the occupants of a building. Regulatory agencies typically have responded to this uncertainty by asking for multiple rounds of sampling over extended time periods to reduce the possibility of failing to identify unacceptable risks, and by recommending building surveys to identify and remove indoor sources before sampling. However, extended sampling periods to assess the potential for VI impacts may be unacceptable in many situations and experience has demonstrated that surveys often miss background sources or that some background sources cannot be removed. This paper presents a test method, Building Pressure Cycling (BPC), that can in a short time frame (e.g., over a weekend) quantitatively characterize the contribution of background sources to indoor air concentrations and determine RME indoor air concentrations due to VI. The method involves measuring indoor air concentrations and building ventilation rates while depressurizing the building, which promotes VI, and while pressurizing the building, which inhibits VI. The RME due to VI alone is determined from the difference between the concentrations measured when the building is depressurized versus pressurized. The concentration measured while the building is positively pressurized represents the contribution of background sources to indoor air. The method has been applied at several commercial and residential buildings and demonstrated to provide reproducible results over multiple days and seasons. These results will be used to demonstrate how the method can identify buildings susceptible to V and improve the selection of appropriate mitigation measures.

05.02.11 Ambient Air Data: Key Data Tool for Risk Management in Vapor Intrusion Assessment in California

C. Peterson, EHS Support / Risk Assessment

A vapor intrusion assessment at a Site in Los Angeles County, California evaluated potential vapor migration pathways associated with affected groundwater beneath the Site. Groundwater quality is affected both regionally and from an adjacent hydraulically upgradient facility. Collection of nested subsurface and sub-slab soil gas, indoor air, and ambient air samples was completed and data were assessed for cancer risks and noncancer hazards for each location using DTSC guidance relevant at the time of sampling. Results from the investigation indicated major risk driving chemicals in indoor air appeared to be consistent with outdoor ambient air constituents rather than vapor intrusion constituents in sub-slab and subsurface soil gas. Ambient air data provided key information for risk management of potential human indoor air exposure to risk drivers from outdoor ambient air versus from soil gas migration from subsurface sources.

05.02.15 Alternative Oral Reference Dose Derivation for Inorganic Vanadium Compounds

M. McCaskill, Michael McCaskill / Health; A. Nair, S. Watkins, PA Dept of Health / Health

The Environmental Protection Agency (EPA) Integrated Risk Information System (IRIS) reference dose (RfD) for vanadium pentoxide is 0.009 mg/kg day^{-1}. There is also an EPA provisional peer-reviewed toxicity value (PPRTV) reference dose of 0.0007mg/kg day^{-1} based on sodium metavanadate exposure in experimental animals. The order of magnitude difference between these two RfDs could have a major impact on state clean-up standards. However, developing an accurate health-protective RfD for vanadium (V) is complicated by its ubiquitous presence at varying concentrations in the earth's crust and multiple valence states in inorganic vanadium compounds. At the request of a Commonwealth of Pennsylvania sister agency, we provided a suggested RfD for vanadium to address the presented challenges. Vanadium toxicity depends on its valence state and solubility. Generally, the toxicity of V increases as the valence state increases (2+ to 5+). Utilizing RfD methods published by the EPA, we reviewed the literature to determine a critical study and critical effect from which a point of departure (POD) could be established. A high-quality critical study was selected, presenting a 5+ valence state V treatment compound, applicable route of exposure, and relevant critical effect in a sensitive receptor. The results presented in the critical study demonstrate that a dose of at least 5 mg/kg day^{-1} of sodium metavanadate, which corresponds to a dosed 2.1 mg/kg day^{-1} of V leads to developmental effects in the offspring of the study rodents. Utilizing the POD of 2.1 mg/kg day^{-1}, we determined the human equivalency dose (HED) to be 0.109mg/kg day^{-1} by applying the rat to human allometric extrapolation. To determine the RfD, uncertainty factors, i.e., LOAEL to No Observed Adverse Effect Level, intraspecies variability, were applied to the HED, resulting in a theoretical RfD for oral exposure to inorganic vanadium to be 0.001mg/kg day^{-1}. Based on our literature review of oral exposure to inorganic V and subsequent RfD derivation, it does not appear that the EPA's IRIS vanadium pentoxide RfD with a critical effect of cysteine changes in the hair of study rodents will be adequately protective of unborn children and neonates particularly for the more toxic valence states of V.

05.03 Bayesian Networks: Applications for Environmental Risk Assessment and Management

05.03.03 Using Directed Acyclic Graphs to Support Causal Interpretations from Data Analysis for Environmental Assessments

J.F. Carriger, U.S. Environmental Protection Agency / Office of Research and Development

Directed acyclic graphs (DAGs) can represent the causal structure of a problem by relating variables through arrows in a cause-effect fashion. When using DAGs in a Bayesian network, conditional probabilities are established for the relationships between the variables. However, these probabilities are often less stable for different contexts than the qualitative relationships in the DAG structure itself. The stability of an appropriately constructed DAG makes it very useful for understanding causal relationships behind the data used for prediction and diagnosis. Recent research has explored how DAGs can be used to better support causal understanding across analytical tasks. When developing statistical models, the strength of correlations are often not directly interpretable in a causal sense. Still, they are regularly interpreted in such a manner. Known biases in causal interpretations can occur from not understanding the causal structure behind the data including unidentified causal associations with the predictors and between the predictors and unmeasured variables. The DAGs provide a platform for making any causal inferences from data analysis transparent. Understanding the data generating process with DAGs can also help to gain insights and prevent erroneous causal interpretations. This presentation will explore how data analysis for environmental assessments can be assisted with the use of DAGs and the limitations. Stylized examples of relevance for environmental assessment will be provided along with classical cases of how DAGs have been used to recognize the causal relationships behind associations. The application of DAGs to analysis of data can enhance causal interpretations and prevent the conflation of influential factors when the structure of the data generating process is identifiable. 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.

05.03.04 Macroinvertebrate Community Structure as Part of a Multiple-Stressor Risk Assessment of the Upper San Francisco Estuary Using the Bayesian Network Relative Risk Model

E.J. Lawrence, Texas State Department of State Health Services, / Environmental Surveillance and Toxicology; S. Elmstrom, E. Whitney, W.G. Landis, Western Washington University / Institute of Environmental Toxicology and Chemistry

The Institute of Environmental Toxicology and Chemistry at Western Washington University is conducting a multiple-stressor, regional-scale ecological risk assessment for multiple endpoints at the Upper San Francisco Estuary, CA using the Bayesian-Network Relative Risk Model (BNRRM). The BNRRM is a quantitative, probability-based approach that calculates complex relationships between ecological variables in a cause-and-effect framework to provide estimates of risk to endpoints. Macroinvertebrates (MI) are an integral part of aquatic ecosystems, playing a key role in food webs and are present in every niche of the aquatic ecosystem. Macroinvertebrate community structure indices are commonly used as indicators for ecosystem quality because changes in macroinvertebrate community structure can reflect effects from chemical and physical stressors on an ecosystem. In this research we use relative taxa richness as a metric to assess stressor effects on MI community structure. We combined MI sampling data, water quality sampling data, and contaminant sampling data from multiple sources and databases. We are using non-metric multidimensional scaling (NMDS) to identify stressors that influence MI community structure and using machine learning algorithms to quantify the probabilistic relationships between identified stressors and relative taxa richness across regional and seasonal scales. These relationships are integrated as a pathway in the BNRRM as part of a larger ecological risk assessment. This approach can serve environmental decision makers in prioritizing mitigation efforts, considering multiple endpoints and potential stressors across spatial and temporal scales.

05.03.06 Reaching the Ideal Weight? Calibration of a Bayesian Network Weight-Of-Evidence Model for Supporting Animal Alternatives to Toxicity Testing

J. Moe, Norwegian Institute for Water Research (NIVA) / Section of Ecotoxicology and Risk Assessment; A.L. Madsen, Hugin Expert; R. Wolf, Norwegian Institute for Water Research (NIVA) / Ecotoxicology and Risk Assessment; S.E. Belanger, The Procter & Gamble Company (retired) / Global Product Stewardship; T. Braunbeck, University of Heidelberg / AquaTox Centre for Organismal Studies; K.A. Connors, Procter & Gamble Company / Global Product Stewardship; M.R. Embry, Health and Environmental Sciences Institute (HESI); K. Schirmer, Eawag, Swiss Federal Institute of Aquatic Science and Technology / Environmental Toxicology; S. Scholz, Helmholtz Centre for Environmental Research / Bioanalytical Ecotoxicology; A. Lillicrap, NIVA / Section of Ecotoxicology and Risk Assessment

Fish Embryo Toxicity (FET) testing has been proposed as an alternative to using juvenile fish in acute toxicity testing, to reduce the number of live animals required for hazard assessments of chemicals. The European Chemicals Agency has recommended the development of a Weight-of-Evidence (WoE) approach for strengthening the evidence from FET testing. While WoE approaches in the past have been largely qualitative, we have developed a Bayesian network (BN) model for using FET data in a quantitative and probabilistic WoE approach. The purpose of this BN model is to predict fish acute toxicity of a given chemical from data on fish embryo toxicity in combination with other types of available information (lines of evidence): quantitative structure-activity relationships (QSARs), toxicity to other species (algae and daphnids), and fish gill cytotoxicity. Here we will focus on new approaches for optimizing the weighting of these four lines of evidence for more accurate prediction of acute fish toxicity. An online web interface to the BN model is being developed within the CEFIC LRI project SWiFT (*Strengthening Weight of evidence for FET data to replace acute Fish Toxicity*; ECO51). A preliminary version of the online tool is available for testing purposes, and demonstrations of this tool can be given upon demand.

05.03.08 A Bayesian Approach for Quantifying Regional Climate Change Risk and Resilience Within the Adaptive Management Framework

M.G. Cains, Indiana University

The historic, coastal city of Charleston, South Carolina, USA is at the front line of climate change impacts. The region's low-lying lands are sources of vulnerability that ultimately expose the city to flooding shocks (e.g., storm surges) and stressors (e.g., sea level rise) caused by climate change. The presented study demonstrates that, when examined within the adaptive management framework, the paired Bayesian Network-Relative Risk Model and Multi-level Risk and Resilience Parameterization Framework produce an integrated risk and resilience assessment of coastal climate change impacts and management options. The integrated risk and resilience approach and applied methodologies incorporate interface dynamics and uncertainty, which allows for the prioritization of management options while accounting for uncertainty. Exposure to shallow coastal flooding, storm surge flooding, and sea level rise inundation was assessed for five endpoints: residences, cultural resources, commerce, critical infrastructure, and fish and wildlife. Resilience was defined as adaptive capacity and parameterized as the base flood elevation of stakeholder-relevant structures within each assessment endpoint category. Fish and wildlife was the most at-risk endpoint and accounted for 25% of the total risk to the assessed endpoints. The southern portion of James Island in Charleston was the most at-risk region with 20% of the total risk to the assessed risk regions. The sensitivity analysis revealed that the calculated risk was more sensitive to the location and vulnerability of a structural endpoint than the adaptive capacity of the structure (i.e., base flood elevation). Additionally, the influence analysis showed that low stressor scenarios produced lower risk scores than high adaptive capacity scenarios. The assessment approach 1) explicitly integrates constituents of wellbeing with attributes of the built and natural environment, 2) identifies endpoints of value and regions of Charleston most at-risk due to projected climate change flooding impacts, and 3) illustrates the utility of Bayesian networks for evaluating the change in risk due to different stressor and management scenarios.

05.04 - Bioinformatics to Inform Cross Species Extrapolations in Regulatory Toxicology: What Tools Are Available?

05.04.01 Using Seq2Fun to Compare Whole-Transcriptome Profiles Across Six Ecological Species

J. Ewald, McGill University / Natural Resource Sciences; P. Liu, McGill University / Institute of Parasitology; N.S. Hogan, University of Saskatchewan / Department of Animal and Poultry Science and Toxicology Centre; M. Hecker, University of Saskatchewan / School of the Environment & Sustainability and Toxicology Centre; D. Crump, Environment and Climate Change Canada / National Wildlife Research Centre; J. Head, McGill University / Natural Resource Sciences; N. Basu, McGill University / Faculty of Agricultural and Environmental Sciences; J. Xia, McGill University Macdonald Campus

Conducting cross-species extrapolation of 'omics data is critical for using toxicogenomics to inform environmental risk assessment. Prior to real-world application, we must have a deep understanding of how whole-transcriptome profiles compare across many species to identify both opportunities and limitations of cross-species extrapolation. This is time consuming and complex, with challenges including replicate genes from genome duplications, one-to-many ortholog mapping, and *de novo* transcriptomes that have hundreds of thousands of poorly annotated transcripts. Here, we introduce Seq2Fun (www.seq2fun.ca), a reference-free and species-agnostic tool for RNA-seq quantification. We demonstrate how Seq2Fun can be used to simplify cross-species comparisons of RNA-seq profiles in a case study. Seq2Fun works by translating reads into amino acid sequences and then directly mapping them to the KEGG database of protein sequences, resulting in a counts file that is expressed in terms of KEGG ortholog (KO) IDs. Because each read is processed individually, Seq2Fun is >120 times faster than *de novo* transcriptome assembly and can be run on a standard desktop computer. Since Seq2Fun maps reads to the same set of KOs regardless of species, the results are well-suited for cross-species comparisons. In the case study, we compare RNA-seq profiles from multiple life stages of six species that were exposed to the same set of eight environmental contaminants (n = 674 profiles; collected as part of the EcoToxChip project, see more at www.ecotoxchip.ca). In the analysis, we 1) identify species and life stage-specific KOs; 2) compute relative conservation of KO expression at the pathway level; and 3) compare transcriptomic responses to the same chemical across different species. In doing so, we show how Seq2Fun reduces barriers for cross-species comparison of whole-transcriptome profiles.

05.04.02 Assessment of Evolutionary Patterns of Tolerance in Aquatic Toxicity Data

A. Coleman, University of Southern California / Department of Biological Sciences; S. Edmands, University of Southern California

Chemical pollution presents one of the greatest threats to the health and functioning of aquatic ecosystems worldwide. Targeted management efforts can reduce the impacts of contaminants on aquatic life, but are constrained by the absence of empirical toxicity data for most contaminants and species. Given that experimental determination of sensitivity is costly and infeasible for many taxa, there is an increasingly urgent demand for tools that can reliably extrapolate toxicity data. Current methods utilize data from surrogate species to predict the tolerance of untested species, but this approach may not be appropriate for distantly related taxa. Here, we examine the evolutionary patterns of sensitivity to twelve chemicals with various toxic modes of action and environmental histories in a taxonomically diverse set of aquatic organisms using a phylogenetic framework. Using multi-gene phylogenetic trees paired with tolerance data, we estimate the phylogenetic signal evident in the data for each chemical, examine taxonomic patterns of sensitivity and explore whether the phylogenetic restriction of tolerance differs between naturally-occurring and synthetic chemicals. Knowledge of the evolutionary patterns of sensitivity may help determine for which taxa and contaminants the use of the surrogate species approach is appropriate.

05.04.03 Developing Functional Extrapolation Approaches Across Species to Inform ERA

C. Rivetti, J. Houghton, G. Hodges, B. Campos, Unilever / Safety and Environmental Assurance Centre (SEAC)

Cross-species extrapolation has been a long-standing in toxicological sciences both in term of environmental safety (e.g. using toxicity data from a reduced number of model species to represent the entire ecosystem) and human health (e.g. using laboratory studies typically from rodents to infer on potential effects on humans). Nevertheless, with the regulatory and ethical landscape demanding an accelerated move towards assessing chemical safety without the use of animals, there is an opportunity for expanding and strengthening this concept beyond its traditional scope using computational tools based on mechanistic knowledge. A comprehensive, evidenced and reliable extrapolation across species would ultimately allow an expansion of the use of the existing data pool which could coherently and more efficiently characterize overall hazard to both, humans and the environment. In this context, leveraging on integrated use of all available data in a Weight-of-Evidence (WoE) approach could serve as a scaffold for a more mechanistically/ pathway-driven testing strategy for complex hazard characterization. Understanding the similarities in physiological processes (i.e. pathways) across species is critical to support and assess and ultimately predict conserved response patterns/metabolic pathways and associated outcomes for certain Modes of Action (MoA). This facilitates a more robust identification of the species at potential risk and ultimately informing Environmental Risk Assessment (ERA) and decision-making. For this purpose, a novel pipeline for functional extrapolation of toxicity effects across species has been developed, which integrates and socializes existing open-source software and genome/pathway databases and provides evidence of the gene conservation and functional coverage across 6 model species by: 1) linking human genes to relevant human pathways; 2) using orthology to assess the conservation across species and 3) retrieving protein domains to infer functionality. The goal is to understand the potential degree of conservation across species covering different branches of phylogeny, starting from identified human perturbed biological pathways in response to chemical exposure. The proposed application within the ERA framework as well as its relevance in supporting decision-making for chemicals with specific MoA will be discussed. The workflow will also be applied to support the decision in a hypothetical safety assessment scenario using a WoE approach.

05.04.04 Bioinformatics for Cross-Species Chemical Susceptibility Prediction and Interpretation of In Vitro Screening Results: Case Study Using a Thyroid Deiodinase Enzyme

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New Approach Methodologies (NAMs) are being developed to reduce animal use and to evaluate greater numbers of chemicals more efficiently. In vitro chemical screening assays can be used to evaluate thousands of chemicals rapidly at lower costs than in vivo assays. Such approaches can specifically target the endocrine system. However, due to varying assay conditions and chemical characteristics, interpretation of in vitro results can be challenging, especially when comparing across species. To better understand results from an in-vitro screening study comparing chemical inhibitory activity between recombinant human and amphibian (*Xenopus laevis*) Type 3 iodothyronine deiodinase (DIO3) enzymes, the putative inhibitors were evaluated using molecular modeling and virtual docking. The DIO3 enzyme inactivates the thyroid hormone (TH) by catalyzing removal of iodine. To function, DIO3 must form a homodimer anchored in the cell membrane. Some chemicals with low in vitro IC50 values matched the native TH-substrate-based pharmacophore and showed affinity for the catalytic site. Other chemicals may be allosteric inhibitors, showing affinity for a location on the DIO3 enzyme outside the catalytic site. However, in a model that included the cell membrane lipid bilayer, many of the chemicals exhibited greater affinity for the lipids than the protein, potentially indicating inhibition through lipid bilayer disruption. In another approach to understand how cross-species variations in protein sequence affect chemical susceptibility, the human DIO3 sequence was modified using in silico site-directed mutagenesis. Single amino acid modifications were made to represent variations in other species at positions critical to enzyme catalytic function. The species-specific amino acid sequence variations were identified using the US EPA Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) tool. These variations were then introduced in the laboratory through recombinant human DIO3 genes inserted into plasmid vectors to produce modified enzymes in cell culture. A suite of inhibitors selected from the previous in-vitro screening were assayed to evaluate the in-silico predictions of chemical susceptibility for each DIO3 variant. These studies establish the basis for a pipeline using bioinformatics and molecular laboratory methods to advance NAMs for cross-species extrapolation. The contents of this abstract neither constitute nor necessarily reflect USEPA policy.

05.04.05 UDP-Glucuronosyltransferase (UGT) in Pinniped Species Can Be Analyzed with the SeqAPASS Tool to Predict Pseudogenes in Other Species

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Differences in xenobiotic metabolism have been identified across species and attributed to the presence or absence of a wide range of phase I, phase II enzymes and transporters. In this context, the ubiquitous phase II UDP-glucuronosyltransferase (UGT) isoforms play a central role in the conjugation and excretion of xenobiotics. UGT activity in hypercarnivores such as the domestic cat (*Felis catus)* is known to be deficient and acetaminophen conjugation in felines has been shown to be slower than that in the domestic dog (*Canis lupus familiaris)* due to the presence of a UGT1A6 isoform pseudogene. This pseudogene is caused by a premature stop codon in exon 1 of the cat UGT1A6 protein. Recently, the northern fur seal (*Callorhinus ursinus*) has been shown to have a UGT1A6 pseudogene and similar UGT activity to the domestic cat. To explore UGT1A6 sequence differences across species, the USEPA Sequence Alignment to Predict Across Species Susceptibility tool (SeqAPASS) tool was used. The SeqAPASS tool allows users to rapidly evaluate available protein target sequence and structural similarity at the primary amino acid sequence, conserved domain(s), and individual amino acid residue levels, to understand conservation and therefore predict chemical susceptibility. Through literature review, a case study was developed to compare the functional dog UGT1A6 to Pinniped species with known functional proteins and known pseudogenes. Using the individual amino acid residue comparison feature in SeqAPASS allowed for hypothesis generation relative to species-specific predictions of possible UGT1A6 pseudogenes. Specifically, an insertion mutation, resulting in a premature stop codon, at aligned amino acid positions 62-64 in the domestic dog was compared to identify UGT1A6 pseudogenes in Pinnipeds. This study demonstrates the utilities of the SeqAPASS tool to rapidly identify the presence of pseudogenes in animal species, generate research hypotheses, and potentially integrate such information in risk assessment to characterize the metabolic basis of species sensitivity to xenobiotics. *The contents of this abstract neither constitute nor necessarily reflect the policies of USEPA and EFSA policy.*

05.05 Current Advances in Bioaccumulation Assessment and Predictive Tools for Nonionic and Nonpolar Organic Chemicals

05.05.01 Probing the Thermodynamics of Biomagnification in Captive Polar Bears by Equilibrium Sampling of Dietary and Fecal Samples

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Biomagnification is the process through which organisms can have higher lipid-normalized concentrations of hydrophobic contaminants in their bodies than are prevalent in the food they eat. The underlying mechanism is a decrease in volume (V) and fugacity capacity (Z) of the food for the biomagnifying contaminants during digestion and assimilation in the gastrointestinal tract. In a proof-of-concept study, we earlier used equilibrium sampling with silicone films to noninvasively derive the thermodynamic limit to a canine's biomagnification capability (BMF_{lim})

by determining the ratio of the *V·Z* products of undigested and digested food. Low contaminant levels prevented the determination of the fugacity (f) or chemical activity of contaminants in food and feces. For polar bears from the Toronto Zoo, mainly fed on fish and seal oil, we were not only able to measure the *Z*-value of diet and feces by equilibrium sampling, but also the increase in the f of native polychlorinated biphenyls (PCBs) upon digestion, providing incontestable proof of the process of gastrointestinal biomagnification. We could also confirm that equilibrium sampling yielded similar *Z*-values for PCBs originally present in food and feces and for isotopically labelled PCBs spiked onto those samples, so that the determination of f even in samples with small size and low contaminant levels becomes possible as long as the concentration of the compound in the sample (C_{native}) can be quantified (i.e. $f = C_{native}/Z_{spiked}$). For zoo bears eating a diet rich in lipids (13.5 %, as fed basis), an observed BMF_{lim} of ~146 indicates a very high biomagnification capability, which can be attributed to reductions in both Z (Z_D/Z_F from 23 to 130) and V (V_D/V_F = 3). Our study highlights the importance of a high fat diet and a high lipid assimilation efficiency (99 %) for achieving high BMF_{lim}. A PCBs uptake efficiency of 98 % indicates that contaminant concentrations in the zoo bears are still far from steady-state, thus resulting in a biomagnification factor ($BMF \approx f_{feces}/f_{food} = 3$) that is much smaller than the BMF_{lim}.

05.05.02 Fugacity Based TMFs Characterize Bioaccumulation of Cyclic Methyl Siloxanes Within a Terrestrial Food Web: Importance of Organism Body Temperature

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This is the first study to investigate trophic magnification of cyclic volatile methyl siloxanes (cVMS) in a terrestrial food web. Concentrations of D4, D5, and D6 and two reference chemicals were measured in air and biota samples of Cooper's hawks, songbirds, and invertebrates from Metro Vancouver, British Columbia, Canada. When derived from lipid normalized concentrations, trophic magnification factors (TMF_Ls) for D4, D5, and D6 were 0.62 (0.11 SE), 0.94 (0.17 SE), and 1.1 (0.23 SE) and not statistically biomagnifying ($p > 0.05$). But D4 was biodiluting ($p < 0.05$). In contrast, TMF_Ls of PCB-153 and *p,p'*-DDE were 5.6 (2.2 SE) and 6.1 (2.8 SE) and statistically biomagnifying ($p < 0.001$). TMF_Ls of cVMS in this terrestrial food web were equivalent to those in aquatic food webs. However, when TMFs were derived on a fugacity basis (TMF_F), incorporating differences in body temperature and lipid composition between organisms, TMF_Fs of all chemicals were greater than corresponding TMF_Ls. Thus, if food webs include endothermic and poikilothermic organisms, we recommend using fugacity based TMFs and including body temperature and tissue composition of organisms to avoid inaccurately characterizing the biomagnification potential of chemicals.

05.05.03 New Perspectives on the Calculation of Bioaccumulation Metrics for Chemicals in Living Organisms

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Today, ready-to-use convenient tools in ecotoxicology to diagnose and predict the accumulation and effects of chemical substances on living organisms, accounting for various exposure situations that are known to be complex (routes of exposure, metabolization processes, etc.) are extremely limited. Among available methods, toxicokinetic-toxicodynamic (TKTD) models are now strongly recommended to describe the link between exposure and effects on individual life-history traits according to time from experimental data collected through standard toxicity tests. In particular, the TK part is used to relate the exposure concentration to the internal concentration within organisms, considering various processes such as accumulation, depuration, metabolism and excretion (ADME). Some regulations (*e.g.*, plant protection products in marketing authorisation applications) require a bioaccumulation test on fish according to OECD Test guideline 305, which consists in an accumulation phase measured by a depuration one. The concentration of the substance within fish is followed over time during both phases thus allowing to model the time course of the exposure within organisms, summarized by bioaccumulation metrics. On a regulation point of view, these metrics are key decision criteria for further estimating the concentration of active substances present in food items of vertebrates. This presentation will illustrate a ready-to-use web tool, $MOSAIC_{bioacc}$ (https://mosaic.univ-lyon1.fr/bioacc) automatically providing estimates of bioaccumulation metrics associated with their uncertainty, as well as the fits of TK models and the estimation of their corresponding kinetic parameters. This tool facilitates the daily work of regulators, but also of any user, who benefits of a freely available and user-friendly on-line interface avoiding investing into modelling technicalities. $MOSAIC_{bioacc}$ allows consideration of the growth of organisms, as well as several uptake routes, and offers the possibility to choose sub-models according to the processes considered. $MOSAIC_{bioacc}$ goes beyond the OECD Test guideline 305 in considering other species than fish (*e.g.*, terrestrial organisms, birds, etc.), and in including biotransformation processes, thus allowing users to easily performed a TK analyses accounting for metabolites. Besides, $MOSAIC_{bioacc}$ was successfully tested with more than 150 TK data sets available in published scientific papers, which highlights its robustness.

05.05.04 Application of an Aquatic Food-Web Bioaccumulation Model in an Urban River for Deriving PCB Remediation Targets for Surface Water, Porewater, and Sediments

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Polychlorinated biphenyls (PCBs) are major contaminants of concern in the Anacostia River, and are responsible for fish-consumption advisories in District of Columbia (DC). The freely dissolved concentration in surface water and sediment porewater are the two thermodynamic drivers for uptake of PCBs in aquatic organisms including fish. This study presents application of a framework for deriving surface water and sediment remediation targets for the Anacostia River using a congener-level approach. Freely-dissolved concentrations of PCBs in surface waters were measured using polyethylene passive samplers, over a period of one year, encompassing all four seasons. Bulk surface sediment PCB measurements and ex-situ measurements of freely-dissolved PCBs in porewater were used to develop site-specific partitioning coefficients. Surface sediment PCB data from existing Remedial Investigation study were used to calculate Surface Weighted Average Concentrations (SWACs) for six sections of the river. These measured data were used to predict steady-state bioaccumulation of PCBs in the aquatic food web, using the software, AQUAWEB, based on the bioaccumulation model of Arnot and Gobas (2004). Congener- and organism-specific apparent organism-water partitioning coefficients and fractional thermodynamic uptake from surface water and sediment porewater, based on application of a deconvoluted version of the Arnot and Gobas bioaccumulation model, were calculated using validated predictions. These parameters are being applied to derive surface water, porewater, and sediment remediation targets to achieve a safe consumption ∑PCB target in fish. These remediation targets, developed for different sections of the river using a congener-level approach, will be compared with those derived using biota-sediment accumulation factors based on total PCBs. The deconvoluted approach considers the orders of magnitude differences in the bioaccumulation potential across the range of PCB congeners. Thus, framework presented in this study can be regarded as a more effective method to utilize expensive congener-level site characterization data that is frequently necessary for baseline, risk, and performance assessments at contaminated sites, and can potentially inform the remedial decision-making process at such sites.

05.05.05 The Application of In-Vitro In-Vivo Extrapolation Using Isolated Perfused Trout Livers Towards High Throughput Screening of Environmental Contaminants

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Bioconcentration factor (BCF) is one of the most common endpoints in chemical risk assessment, informing bioaccumulation status. While BCF as determined from in-vivo whole fish exposures is still considered the gold standard to inform this criterion, there is growing concern in academia, governments, and industries about the suitability and reproducibility of this test, especially for chemicals that are biotransformed. Alternative approaches using in-vitro biotransformation assays based on hepatocytes or liver sub-cellular fractions in combination with in-vitro in-vivo extrapolation (IVIVE) models have been developed as potential replacements. However, extrapolation to BCF is complicated by confounding factors, e.g., extrahepatic biotransformation and quality issues with experimental BCFs. Therefore, there is a need for an ex-situ model at an intermediate level of biological organization. A recently developed method is that of the rainbow trout isolated perfused liver, seeking to reduce uncertainty in IVIVE of clearance rates of chemicals. The present study seeks to obtain hepatic clearance data of four environmental contaminants of interest within the isolated perfused trout liver and cross-validate with prior standardized in-vitro methods. Livers of sexually immature rainbow trout were cannulated via the hepatic portal vein and perfused for six hours with a physiological buffer spiked at varying concentrations of pyrene, 4-nonylphenol, deltamethrin, and methoxychlor. Afferent and efferent samples were taken in 15-minute intervals across the perfusion period. Samples were analyzed using high-performance-liquid-chromatography with fluorescence detection (HPLC-FLD) and gas-chromatography (GC) to calculate hepatic extraction fraction and clearance. Results demonstrate that this experimental method can be used to validate IVIVE models, as illustrated by the excellent fit of predicted versus measured hepatic clearance values. This study has the potential to settle an important debate in this field and enables scientists to focus on other factors to allow for confident predictions of bioconcentration in fish.

05.05.06 CBD = Cannabinoid Biotransformation Discoveries: Understanding Cannabinoids Biotransformation Using In Vitro Metabolism Technologies (OECD TG 319B)

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Acceptance and legalization of industrial hemp, cannabinoids (e.g. CBD and THC), and marijuana is rapidly growing in North American countries and around the world. In many instances, new regulations will be considered and implemented after the fact. While CBD has brought to light benefits in treating human and domesticated animals (e.g. dogs and cats) ailments, there is a notable lack of discussion around the impact that these compounds will bring on the environment and freshwater supplies in many areas worldwide. There is a need for more research to fill the information gaps that haven't been covered due to the previous illegal status of these compounds. Biotransformation of chemicals using *in vitro* technologies is increasing and essential for bioaccumulation prediction and chemical risk assessment. Likewise, *in vitro* technologies to test chemicals for risk assessment is increasing due to the benefit of reducing the number of animals used for testing, cost and time of experimentation. *In vitro* metabolism technologies can be applied to test metabolism, determine a Bioconcentration Factor (BCF) when *in vitro* to *in vivo* models are available, and identify metabolites of the different cannabinoids. The objective of our initial study is two fold. First, cannabinoid metabolism, particularly CBD, will be studied across multiple species by exposing liver S9 fractions (OECD TG 319B) from trout and other fish species and compare metabolic rates to humans, rats, cats, and dogs. Rainbow trout, carp, bluegill, largemouth bass, alligator gar and Gulf killifish were acclimated to laboratory conditions and used in the preparation of liver S9 fractions. Mammalian liver S9 fractions were obtained from commercially available sources. Secondly, with the metabolic rates determined in multiple fish species, we can begin the discussion of the implications of cannabinoids and their presence in the environment as it relates to ecological chemical risk assessment for human and environmental health.

05.05.07 Near Real-Time Prediction of PAH Bioaccumulation in Individual Oil Exposed Oysters Using Antibody-Based Biosensor Technology

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Sessile, filter feeding bivalves such as oysters can readily bioaccumulate environmental contaminants like polycyclic aromatic hydrocarbons (PAH) freely dissolved in water. With limited metabolic capacity, oysters lack an efficient means to excrete PAH. Following major PAH contamination events such as oil spills, rapid screening of PAH levels in highly valuable seafood such as oysters is critical to minimize lengthy fishing closures. Established rapid screening methods after a spill event such as sensory analysis (i.e. sniff-testing) are non-quantitative and met with distrust by the public. Reliable PAH quantification methods such as gas chromatography-mass spectrometry (GC-MS) are time consuming and expensive, resulting in a significant time lag for data analysis. A highly sensitive antibody-based biosensor method has been developed to measure total 3-5 ring PAH concentration in small volume (1-2 mL) aqueous samples in minutes. Concentrations measured using this method correlate well with conventional GC-MS analysis. With these features, the biosensor has shown great promise as a fast screening tool to measure PAH contamination in oysters and has been utilized to rapidly and inexpensively measure PAH concentration in oysters throughout an entire river watershed in southeast Virginia impacted by legacy PAH contamination. To better understand the applicability of this method in oil spill response, a laboratory study was conducted in which adult *C. virginica* oysters were exposed to the water-accommodated fraction of heavy crude oil for 3 days at a 2.5 ppb dose followed by a 14-day depuration period in uncontaminated water. Throughout the study, animals were sampled to compare the ability of the biosensor to track changes in PAH concentration overtime to that of conventional body burden analysis by GC-MS. The biosensor measurements showed the rapid uptake of PAH during the dosing period and then traced the depuration of the contamination by the oysters during the two-week period. With the ability to measure concentrations in near real-time at a fraction of the cost compared to conventional GC-MS, biosensor technology could serve as a powerful screening tool to prioritize affected sites during time-sensitive situations like oil spill response. Following a spill, rapid measurement of PAH levels would be highly valuable to local communities and other stakeholders whose livelihood depend on the fishery.

05.05.08 Population and Sex Differences in the Biotransformation of Polycyclic Aromatic Hydrocarbons (PAHs) by Gulf Killifish (Fundulus grandis) with Different Exposure History

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Gulf killifish (*Fundulus grandis*) populations inhabiting the Houston Ship Channel (HSC), Texas, USA, have been recently documented to be pollution-adapted. Although not fully elucidated, one particularly important aspect of their adaptation involves the reduced inducibility of the aryl hydrocarbon receptor (AhR) and, potentially, the alteration of major biotransformation pathways. In the present study, we employed a modified

Organization for Economic Cooperation and Development (OECD) 319-B test guideline to explore population and sex-related differences in the hepatic biotransformation of six polycyclic aromatic hydrocarbons (PAHs) in *F. grandis* populations with different exposure histories. Pollution-adapted *F. grandis* showed significantly lower hepatic clearance of PAHs than non-adapted fish, especially for high molecular weight PAHs, and with pollution-adapted females presenting the lowest clearance. The activity characterization of selected phase I biotransformation enzymes revealed that the basal activity of cytochrome P450 1A1 (CYP1A1), fundamental in the biotransformation of PAHs, was significantly lower in pollution-adapted fish, especially in females, which showed the lowest activity. Contrarily, basal CYP2C9-like activity was significantly higher in pollution-adapted fish. On-going research has also elucidated reduced cytotoxicity and CYP1A1 activity in primary hepatocytes of pollution-adapted *F. grandis* exposed to a PAH mixture. This experimentation highlights the importance of exposure and evolutionary histories in shaping organisms' responses to pollution.

05.05.09 Pre-Remedial Monitoring of PCB Bioaccumulation in Mummichogs and Banded Killifish in the Anacostia River Watershed

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The District of Columbia Department of Energy and Environment (DOEE) is investigating contamination of the Anacostia River in Washington, DC. through the Anacostia River Sediment Project (ARSP). In 2020, DOEE issued an Interim Record of Decision. The major goals are to clean up sediment polychlorinated biphenyl (PCB) hot spots and thus decrease PCB concentrations in game fish that people eat. To establish a 3-year pre-remedial baseline, we collected small forage fish (mummichogs (*Fundulus heteroclitus)* and banded killifish (*F. diaphanus*)) from multiple sites in six tributaries, the mainstem, Kingman Lake (an oxbow within the river), and the Potomac River as a reference. This approach takes advantage of their < 0.5-mile home range, 3-year lifespan, and widespread abundance. We developed a linear model to evaluate covariates for comparisons across locations. Lipid content was a significant covariate as was species (in a combined species model). PCB concentrations were elevated (medians of 321 to 1055 ppb wet weight) in both species from two Lower Beaverdam Creek (LBC) tributary locations relative to other locations. The pattern of PCB homologs (enriched in di-, tr-, and tetra- congeners) in LBC fish suggested a different (and more recent) source of PCBs from that in all other locations. This study and others have confirmed LBC as a current source of loading of bioavailable PCBs to the tidal Anacostia. Fish from the Northwest and Northeast Branches, which together contribute about 80% of the sediment load, had total PCB concentrations < 100 ppb, similar to most Potomac locations. Low density polyethylene (LDPE) passive samplers deployed in the water column for two months at the same locations gave similar PCB concentration patterns; predictions of total PCB concentrations in fish were within a factor of 3 of those measured in this study. At the congener level, 53% to 79% of the PCB congeners predictions agreed with measured concentrations within a factor of 4. Repeating surveys about every three years will allow DOEE to measure progress due to remedial actions, source control, and natural attenuation.

05.05.10 Interpreting Variance to Evaluate Laboratory Benthic Bioaccumulation Data: Three Case Studies From the Laurentian Great Lakes

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Evaluating PCB bioaccumulation risk from sediment to aquatic receptors typically first considers the bulk concentrations in sediment and the associated residues bioaccumulated in infaunal benthic organisms exposed in the laboratory. When extrapolating to exposures in the field, however, such data are often assumed to represent a direct relationship between sediment and receptors, without considering other factors contributing to bioaccumulation or its variability. Exposure to low-level sediment PCB concentrations particularly produces variability in laboratory-generated benthic tissue residues that encumbers clear data interpretation. This presents a challenge for determining whether a minor increase in bioaccumulation from test sediment relative to reference sediment indicates a potential for adverse effects to the aquatic ecosystem. We present a weight-of-evidence approach for evaluating such outcomes that strengthens interpretation of statistical differences identified in laboratory bioaccumulation datasets. In comparison to the field, fewer factors contribute to bioaccumulation variability in standardized laboratory tests, thereby producing precision among test replicates that result in statistically significant differences among test sediments with relatively similar mean bioaccumulation estimates. Evaluating the magnitude of difference—and the risks associated with the expression of such a difference on the system of study—is therefore a key interpretive lens based on standard statistical practice. Three case studies of PCB bioaccumulation from Great Lakes basin sediments are used to demonstrate how evaluating variance and magnitudes of difference provides practical interpretation of benthic bioaccumulation results. These cases show that a benchmark magnitude of difference of two is robust when considering the variance of benthic bioaccumulation observed across a narrow range of bulk sediment concentrations, across four years sampling the same sediments, and across paired laboratory and field measurements. Collectively, this highlights the utility of directly evaluating the temporal and random variance introduced along bioaccumulation pathways and through extension to ecological endpoints. We demonstrate that bioaccumulation to the benthos from Great Lakes sediment exhibits enough variance on its own that employing a benchmark factor of two provides judicious interpretation for determining when adverse impacts to the aquatic ecosystem are unlikely.

05.05.11 Validation of the Hyalella azteca Bioconcentration Test (HYBIT)

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Bioconcentration factors (BCF) for regulatory purposes are conventionally determined experimentally by aqueous exposure bioconcentration fish tests according to OECD test guideline (TG) 305. Fish bioconcentration studies are time consuming, expensive, and use vertebrate organisms in the range of 100-200 organisms per study. The availability of alternative methods may help to reduce the use of fish for BCF testing. The *Hyalella azteca* Bioconcentration Test (HYBIT) provides a non-vertebrate alternative for fish bioconcentration studies. The suitability of *H. azteca* as an alternative test organism for bioconcentration studies was recently investigated. Eighteen substances of different hydrophobicity (log K_{OW} 0.7 - 7.8) were tested under flow-through or semi-static conditions to determine steady-state and kinetic bioconcentration factors (BCF_{SS} and BCF_K). It has been shown that the BCFs obtained from bioconcentration studies with the freshwater amphipod *H. azteca* correlate significantly (r^2=0.69) with fish BCF values described in the literature. Thus, H. azteca BCF values can be assessed in accordance with the standard B criteria, e.g. BCF > 2000 (REACH), and thereby enable the prediction of B or non-B classification in the standard fish test. Generally, technical (smaller-scale test systems) and economic reasons (less time-consuming, more cost-efficient) favour the use of *H. azteca* over fish in bioconcentration studies. In addition, significantly smaller amounts of test substance are required compared to the flow-through test with fish. A protocol for carrying out bioconcentration tests with the aquatic invertebrate species *H. azteca* under standardized conditions has been developed as part of the project CEFIC-LRI ECO40. This protocol includes both, the flow-through and semi-static test design. Validation was needed to confirm the transferability of the test protocols and to prove the reproducibility of the results obtained in order to support the development of a new OECD TG. For this purpose, a multi-laboratory ring trial involving the HYBIT was carried out in 2020. The ring test has confirmed the high potential of the *Hyalella azteca* Bioconcentration Test (HYBIT) to be used as a non-vertebrate alternative for bioconcentration studies. The transferability of the test protocols (semi-static and flow-through approach) as well as the reproducibility of the results obtained was proven supporting the development of a new OECD TG.

05.05.12 Introducing BAT ver.2.0: A Weight-of-Evidence Framework for Bioaccumulation Assessment

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Bioaccumulation (B) assessments using various methods, data, metrics and classification criteria are routinely conducted as a part of chemical regulatory programs. However, the myriad of bioaccumulation metrics and threshold values combined with the uncertainty and variability in measured data can make decision-making challenging. In October 2018 the Bioaccumulation Assessment Tool (BAT) ver.1.0 was released to help facilitate transparency and consistency in B assessments. Based on stakeholder feedback, improvements to workflow and flexibility are now implemented along with the ability to include data for aquatic invertebrates and relevant in vivo laboratory data for rodents. The BAT ver.2.0 (2021) guides a user through the process of collecting and generating various lines of evidence (LOE) required for assessing the bioaccumulation of neutral and ionizable organic chemicals in aquatic organisms and homeotherms (mammals). The user may enter various B metrics (i.e., LOE) for aquatic organisms such as in vivo laboratory BCFs and BMFs, field BAFs and field TMFs. Additionally, in vivo, in vitro and in silico biotransformation rate data which are used by the BAT along with partitioning data to predict various B metrics using mechanistic mass balance models may also be entered. These in silico B metrics can then be compared to the empirical data entered by the user. B metrics relevant to air-breathing animals (e.g., field BMFs) can also be entered by the user along with biotransformation rate data. As with aquatic organisms, biotransformation data are used by the BAT to predict B metrics of interest. To evaluate each LOE, the BAT includes Data Evaluation Templates (DETs) developed from standardized testing guidance. As illustrative examples, the BAT ver.2.0 was applied to assess the bioaccumulation potential of hexachlorobenzene (HCB) and β-hexachlorocyclohexane (β-HCH) and successfully captures key similarities and differences in the behaviour of these two chemicals in the environment. In summary, the BAT ver.2.0 provides a formal weight of evidence (WOE) framework for bioaccumulation considering both aquatic and terrestrial data sources consistent with OECD WOE principles. It facilitates the implementation of policy objectives in chemical assessment and management, regardless of jurisdiction. The BAT ver.2.0 is now publicly and freely available online and can readily be used by interested stakeholders from academia, industry and the regulatory community.

05.05.13 Riparian Spiders: Biosentinels of Polychlorinated Dibenzo-P-Dioxin and Dibenzofuran Contaminated Sediment

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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.

05.05.14 Bioaccumulation of Phenolic Benzotriazoles in the Benthic Freshwater Amphipod Hyalella azteca

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Phenolic benzotriazoles are an important class of UV absorbers (UVs). However, researches have demonstrated potential bioaccumulation of these compounds in aquatic organisms and birds. The identification and scientific assessment of compounds that bioaccumulate in organisms and biomagnify in food webs play a key role within the regulatory chemical safety assessment. The bioaccumulation potential of compounds is commonly expressed as bioconcentration factors (BCF), determined in flow-through studies with fish according to OECD 305. However, fish bioconcentration studies are time consuming, expensive, and use many laboratory organisms in the range of 100–200 organisms per study. An alternative test system for BCF tests using the freshwater amphipod *H. azteca* (HYBIT) was recently suggested. Hyalella BCFs show a strong correlation with fish BCF values. However, UVs are difficult to apply due to their very low solubility in water. In this study we tested the suitability of the new test system for testing the bioaccumulation potential of UVs. Three phenolic benzotriazoles (UV 234, UV 328 and UV 329) which are highly hydrophobic (log Kow >6) were tested following the HYBIT flow-through protocol. As expected only very low exposure concentrations (≤1µg/L) were reached. Nevertheless, the measurement of substance concentrations in test water and *H. azteca* samples collected during the uptake and depuration phase was still possible and allowed the determination of BCF_{SS} and BCF_{kin} values for the three compounds. The BCF studies showed that the new test system using a non-vertebrate organism is also suitable for the testing of phenolic benzotriazoles and may thus help to identify UV compounds that bioaccumulate in aquatic organisms.

05.05.15 Expanding the Applicability Range of the Generator Column Technique to Measure the Octanol-Air Partition Coefficient of Volatile Substances

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Physical chemical properties such as equilibrium partition coefficients, vapour pressures and solubilities are widely used to understand, predict and assess the fate of organic chemicals in our environment. A logarithmic octanol-air partition coefficient (log K_{OA}) of 5 has been suggested as the threshold for identifying organic compounds that are subject to efficient respiratory elimination in air-breathing organisms. Substances with a log K_{OA} below this threshold are not considered bioaccumulative in air-breathing organisms. Current K_{OA} prediction techniques are sufficiently accurate and precise to classify chemicals with predicted log K_{OA} values outside the range of 4 and 6 as either non-bioaccumulative (log K_{OA} < 4) or potentially bioaccumulative (log K_{OA} > 6). For more volatile compounds (log K_{OA} < 4), the K_{OA} can be measured using static techniques, such as the variable phase headspace technique. The dynamic generator column technique has been shown to work very well for chemicals with less volatile compounds with a log K_{OA} greater than 6. However, there is a relative dearth of K_{OA} measurement in the vicinity of the threshold for respiratory elimination, presumably because this threshold falls at the edge of the applicability range of both static and dynamic techniques. In this work we explore how the applicability range of the generator column technique for measuring K_{OA} can be expanded to more volatile chemicals by modifying the air flow rate, the duration and temperature range of the measurements, and the concentration of the chemicals in the octanol saturated column.

05.06 Ecological Risk Assessments for Contaminants of Emerging Concern

05.06.01 Prioritizing Contaminants of Concern in the Fraser River Watershed: A Risk-Based Evaluation for Outmigrating Juveniles and Returning Adult Salmon

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The Fraser River watershed covers an area of approximately 340,000 km^2, has a mean freshwater discharge of 27,000 m^3s^{-1} and is home to 54 unique spawning populations of salmon, including 19 Chinook (*Oncorhynchus tshawytscha*) populations. Fraser Chinook provide 80-90% of the food source consumed by Southern Resident killer whales (SRKW, *Orcinus orca*) in the spring and summer. Over 90% (15/16) Fraser Chinook populations assessed by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) are at risk. Extensive forestry, agricultural, industrial and urban activities take place in the Fraser Valley that expose early life stages of emigrating salmon and returning adult salmon to a mix of legacy and emerging contaminants that include PCBs, pesticides, pharmaceuticals and personal care products (PPCPs). Many of these contaminants can elicit adverse health effects in vertebrates, including endocrine disruption and reproductive effects. However, there is limited information on the nature of contaminants discharged into British Columbia's salmon habitat and their associated effects, hampering solution-oriented opportunities for natural resource managers and stakeholders. Surface water samples were collected monthly (2018-2020) from seven urban and semi urban sites in the Fraser River watershed, and one site in the Serpentine River, a lower discharge river that flows directly into SRKW critical habitat. Samples were analyzed for over 800 contaminants in order to prioritize contaminants of concern to outmigrating juveniles and retuning adult salmon, with a special focus on Chinook salmon. Measured chemical concentrations were compared to water quality guidelines for the protection of aquatic life and chemical-specific biological activities determined in high-throughput (ToxCast) in vitro assays. A risk-based evaluation using a combination of toxicity quotients and exposure activity ratios was performed to prioritize contaminants for long-term monitoring and to identify chemicals suspected of posing a potential risk to salmon. This study is the first step toward a comprehensive risk-based evaluation for contaminants of concern to salmon in the Fraser River. Results will support the Government of Canada's *Whales Initiative* in its quest to identify contaminants of greatest concern to Chinook salmon and to guide SRKW recovery efforts.

05.06.02 A Framework for Prioritizing Chemicals in Retrospective Ecological Assessments: Application to a Great Lakes Watershed

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Anthropogenic activities related to industrialization, urbanization, and agriculture have resulted in frequent detection of poorly understood contaminants of emerging concern (CECs) across Great Lakes tributaries. Thus, there is a need to identify CECs of higher and lower ecotoxicological concern to help focus risk assessment and regulatory efforts. Here we present a weight-of-evidence framework developed to retrospectively prioritize chemicals detected in contaminated watersheds. The framework is based on a weight-of-evidence approach incorporating consideration of *detection characteristics* (spatial frequency, temporal frequency, environmental distribution), *environmental fate* (persistence, bioaccumulation, biomagnification), *ecotoxicological potential* (exceedance of water quality, *in vivo*, and/or *in vitro* toxicity benchmarks), and *effect covariance* (covariance with effects in caged fish studies, random-forest regression analyses). To demonstrate the overall approach, this framework was used to prioritize organic contaminants detected within the Milwaukee River watershed (Milwaukee, WI). Chemical prioritization was carried out using *in vivo*, *in vitro*, and analytical data generated from Milwaukee watershed caged-fish studies in 2017 and 2018, and chemical-specific data collated from USEPA databases (CompTox Chemicals Dashboard, ECOTOXicology Knowledgebase, ToxCast database) and/or estimated using quantitative structure-activity relationships. Six of 83 CECs detected within the Milwaukee Estuary AOC were identified as high priority, 12/83 were low priority, and 10/83 were significantly data limited requiring further investigation for prioritization. The remainder of the detected chemicals had moderate priority scores. Overall, this study presents a flexible effects-based weight-of-evidence strategy that can be employed for CEC prioritization, and highlights several chemicals of ecotoxicological interest within the Milwaukee Estuary AOC. *The contents of this abstract neither constitute nor necessarily reflect USEPA policy.*

05.06.03 Risk-Based Prioritization of Contaminants of Emerging Concern Detected in Great Lakes Tributaries, 2010–2018

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As part of the Great Lakes Restoration Initiative (GLRI), extensive contaminant monitoring, including 830 unique compounds, was conducted in Great Lakes tributaries over the course of eight years. This monitoring supported the GLRI's aim of identifying significant sources and effects of new toxics and assessing their potential impacts on Great Lakes fish and wildlife. To put monitoring results into context, data regarding potentially hazardous concentrations of substances detected, including contaminants of emerging concern, were assembled from publicly accessible sources. The sources queried included published water quality standards and screening values, data from peer-reviewed studies compiled in the ECOTOX knowledgebase (https://cfpub.epa.gov/ecotox/) and bioactive concentrations in ToxCast assays, and/or Quantitative structure-activity relationships (QSARs). Among the 830 unique contaminants monitored, 420 were detected in surface water. Biological effect concentrations from ECOTOX or ToxCast were available for 300 of the surface water detected compounds (71 percent). A two-dimensional prioritization that considered detection frequency for individual contaminants and toxicity quotients or exposure activity ratios (i.e., ratio of detected environmental concentration to biological effect concentration) was used to group chemicals based on their relative priority for management action, additional monitoring, or research. Examples of high-priority chemicals identified included 2,4-dioxychlorophenoxy acetic acid (2,4-D), naled, methomyl, azoxystrobin, benzo[b]fluoranthene, pyrene, and a number of herbicide degradates. Several steroidal compounds exceeded biological effect benchmarks, but were infrequently detected, suggesting more site-specific concerns. Just as importantly, over 65 percent of the detected chemicals were ranked as relatively low priority based on available exposure and effects data. Priorities based on the individual lines of evidence are being integrated to provide an overall ranking, and the 120 surface water contaminants for which effect benchmarks were unavailable have been prioritized for collection of toxicity data based on overall detection frequencies. *The contents of this abstract neither constitute nor necessarily reflect official policy of the agencies and organizations involved in this research.*

05.06.04 The Prioritization of Contaminants of Emerging Concern in Regional Waters Utilizing Novel Toxicological Measures

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There is a wide range of anthropogenic chemicals in the marine waters of the Puget Sound, some of which are likely to affect the health of important and endangered marine species. Many of the chemicals that are present are the same that we use in daily activities, including pharmaceuticals, plasticizers, flame retardants, and transport-related chemicals. They enter the greater Puget Sound watershed through common use and then are brought into the marine waters through stormwater runoff or wastewater treatment system effluent. We have measured hundreds of chemicals in the water, sediment, and biota. There is a recognized need to prioritize these chemicals to focus management activities and regulatory response. This need has been explicitly identified in management plans (e.g., the Toxics in Fish Implementation Strategy developed the Puget Sound National Estuary Program) and regulation (e.g., the Washington State Pollution Prevention for Healthy People and Puget Sound Act). One way to do so is based on biological relevance, that is to focus on those chemicals with the highest likelihood to cause harm to fish and wildlife, or the humans that eat them. We approach this generally by comparing levels of occurrence in the environment (environmental concentrations) with effects levels (the concentrations which may adversely affect an organism). However, there is a general lack of robust ecotoxicological information. A novel source of data is the Tox21 program, which performs high throughput assays to understand the potential activity of chemicals against important biological receptors and pathways. This can provide useful benchmarks for evaluating if a chemical has the potential to cause harm. In this work we briefly describe the status of CEC occurrence in the Puget Sound, based on a range of opportunistic sampling results. And then provide the results of the prioritization effort whereby the occurrence data was compared to multiple measures of toxicity. The results and implications will be discussed.

05.06.05 Fate and Effects of Microplastics in a Wastewater Treatment Plant in the Western Cape, South Africa

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Municipal wastewater treatment plants (WWTP) effluent discharges are considered as a major contributor of microplastics (MPs) into the aquatic environment. They receive wastewater containing primary and secondary MPs from anthropogenic sources. The Western Cape WWTPs collect sewage from both industrial and residential areas in a combined sewer system. One of the WWTPs was investigated for microplastics at different treatment stages to ascertain the point in the treatment process at which MPs are removed. Samples were characterized in terms of colour, shape, size, and polymer composition using the microscopy and Fourier Transform Infrared spectroscopy. The possible ecological risks of the obtained WWTP microplastics on exposed freshwater biota were investigated using *Daphnia magna*, *Raphidocelis subcapitata* and *Tetrahymena thermophila* as test models for ecotoxicological assessment. This study provided an insight into the fate of microplastics in the WWTP process and the possible ecotoxicological effect(s) of MPs.

05.06.06 The Development of a Skeletal Biomarker of Environmental Pollutant Exposure in American Minks

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The release of pollutants by the oil sands industry and pulp and paper mills has been an ongoing environmental concern for decades. Such toxins have been linked to declining reproductive and skeletal health in wildlife species, as they have known endocrine disrupting properties that interfere with those hormones responsible for proper reproduction and bone development. As such, declining population sizes as a result of pollutant exposure has been correlated with altered bone health in mammals. For the purposes of environmental monitoring of industrial activity, the development of a biomarker of pollutant exposure would be beneficial to assess pollution impacts on wildlife populations. Therefore, the purpose of this research is to identify this biomarker in the American mink based on measures of their bone health. This subaquatic apex predator has a small home range, is abundant in North America and is already trapped for commercial purposes, making it an ideal bioindicator species. Mink from several regions were collected: the Athabasca Oil Sands Region (in Alberta), an area surrounding a pulp and paper mill in Quebec, and a region isolated from industrial activity in New Brunswick. From each mink, four bones were collected: the right hindlimb femur, baculum (for males), cervical and thoracic vertebrae. Bones were evaluated for biomechanical parameters through a series of destructive and non-destructive tests, to quantify key cortical and cancellous bone structural and material properties. These included: dimensional analysis, three-point bending of long bones, axial compression of vertebrae, and micro-Computed Tomography (for internal geometry and density measures). Subsequently, a comparative assessment of bone measures among the three locations is being performed. Combined with toxicology reports of pollutant exposure provided by ECCC, statistical analyses were used to compare the bio-mechanical measures with contaminant levels, and compared among the three source locations. A bone biomarker identified through this research will provide a simple and reliable method for determining whether there are unsustainable levels of pollution in these regions across North America, and to track pollution effects over time. In this way, the protection and preservation of wildlife can be prioritized, through developing environmental regulations that consider subtle health effects on species.

05.06.07 Field Evidence of Wild Bird Exposure to Fludioxonil and Extrapolation to Other Pesticides Used as Seed Treatments

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This study was conducted to determine the exposure of wild birds to pesticides via consumption of treated winter wheat seeds. The study took place on three different fields in southern UK over the two weeks following the sowing day in September/October 2020. We recorded the density of seeds left on the soil surface, bird abundance in the fields, and the rate of consumption of pesticide-treated seed by different bird species using camera traps. The seeds were treated with the fungicide fludioxonil, and we calculate the percentage of a LD50 ingested by each bird species in a single feeding bout, and if they were to eat treated seeds exclusively for one day. Additionally, we conduct similar calculations for 19 other pesticides commonly used as seed treatments, assuming the same rate of consumption as for fludioxonil-coated seeds (i.e. no positive or negative effects on palatability compared to fludioxonil). All three fields contained grains on the soil surface, with an average of 7.13 seeds/m2 on sowing day. In total, 1,465 granivorous birds spanning eighteen different species were observed in the fields, with 11 species filmed eating the seeds. Fludioxonil appears to pose a low risk to birds, for the maximum amount ingested by a bird in a single feeding bout comprised 0.2% of the LD50 (for chaffinch, Fringilla coelebs), rising to 1.14% if a bird consumed exclusively treated seeds for one day. Analysis of a further 19 pesticides commonly used as seed dressings suggests that the neonicotinoid insecticides imidacloprid, clothianidin and thiamethoxam, the pyrethroid insecticide tefluthrin and the fungicide carboxin represent the highest risk for granivorous birds. For example, chaffinch could consume 63% of LD50 of imidacloprid in a single feeding bout, and 370% in a day. Rook (Corvus frugilegus), carrion crow (Corvus corone) and wood pigeon (Columba palumbus) were all predicted to exceed their LD50 for imidacloprid in a day of feeding. Fringilla coelebs was also predicted to receive >20% of the LD50 if it fed for a day upon seeds treated with tefluthrin or carboxin, likely to be sufficient to impact upon health. Further investigation is clearly required to determine whether seeds treated with these other pesticides are consumed as readily as those treated with fludioxonil, as if so this is likely to cause mortality and impaired fitness.

05.06.08 Hazard Assessment of Herding Agents to Commercially Valuable Species of Atlantic Cod (Gadus morhua)

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Oil spill response still seems challenging considering the extent of threats to marine ecosystems and potentially available oil spill response techniques. After an oil spill, the oil spreads on the water surface, creating a thin slick which can present challenges for efficient removal and recovery. In situ burning is an efficient means of removing oil from the water surface, provided the oil slick is thick and contained. Herders reduce the spread of an oil slick by changing the balance of interfacial forces acting on the edge of the oil and decreasing the oil-water surface tension, which makes a thicker oil slick over a smaller area. SilTech OP-40 and ThickSlick 6535 are listed as herding agents on the National Contingency Plan product schedule for consideration for use in the US waters. While evaluated in standard lab tests for regulatory approval, data on the toxicity of SilTech and ThickSlick to marine biota is limited, particularly for commercially important species. Atlantic cod (*Gadus morhua*) inhabit the waters of the Northern Atlantic Ocean and have pelagic embryos. Within Canada, it is labelled as threatened species due to overfishing, which provides a rationale and highlights the importance for investigating the potential impact of herders on it. The toxicity of SilTech and ThickSlick to cod embryos was investigated in this study. Pre-hatch cod embryos were exposed for 24 hrs to control seawater (0.22 µm) and five concentrations of SilTech and ThickSlick with 3 replicates per concentration and 10 cod embryos per replicate. The toxicity assessment was conducted at 24 hrs. The live unhatched embryos were transferred to 0.22 µm filtered seawater and monitored until hatch or mortality. Live fish were imaged and scored using a modified blue-sac disease (BSD) index. Water quality parameters were measured at pre- and post-exposure. The results showed that SilTech is more toxic than ThickSlick, with 50% effect concentration (EC50) for hatching of 6.8 mg/L (nominal concentration) and 402.7 mg/L, respectively. The BSD presentation was a more sensitive endpoint than hatching with EC50s of 4.8 mg/L for SilTech and 94.8 mg/L for ThickSlick. The results of this study provide information for decision making under Net Environmental Benefit Analysis (NEBA) when considering the potential use and selection of specific herding agents for oil spill response.

05.06.11 Comparison of Ecological Screening Levels for Per- and Polyfluoroalkyl Substances in Wildlife

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One of the key components of an ecological risk assessment (ERA) is the use of Risk-Based Screening Levels (RBSLs), which provide risk assessors a strategic, resource-effective approach to better understand potential ecological risk at contaminated sites. For contaminants such as metals and legacy organic compounds, there are well known compendiums of ecological RBSLs, however RBSLs may not be as readily available for emerging contaminants. Due to persistence in the environment, water solubility, and subsequent high volume and or mass of usage and release of materials, per- and polyfluoroalkyl substances (PFAS) are becoming a widespread risk assessment concern. Perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) have sufficient toxicity data to develop ecological screening levels; however, toxicity data is limited for a number of other PFAS. Based on available data, ecological screening levels have recently been developed for wildlife receptors (birds and mammals), aquatic life (fish and aquatic invertebrates), terrestrial invertebrates, and plants. Available RBSLs for wildlife were reviewed to better understand the range of values, the underlying assumptions and methodologies that lead to variation in ecological RBSLs, and overall generalizations with regard to receptors that will most likely influence decision making at PFAS sites. Avian and mammalian wildlife are considered to be more at risk to PFAS, and bioaccumulation in terrestrial and aquatic food webs can lead to higher exposure; therefore, the most important ecological PFAS risk issues at most sites are likely to be associated with risks to vertebrate wildlife associated with dietary intake, rather than direct contact (e.g. aquatic life, plants, soil invertebrates). Similar to human health, ecological RBSLs may vary widely based on endpoint selection and methodology. As regulatory agencies begin adopting RBSLs for use in ERA, it is important to understand what these values are based on. Given the ongoing development of ecological risk model parameters and approaches for PFAS (i.e., uptake factors, toxicity reference values, mixture toxicity algorithms, etc.) development and refinement of RBSLs is going to continue and include additional PFAS; therefore, it is valuable to understand the impact these parameters and approaches can have on determining risk.

05.06.12 Using a Probabilistic Approach in an Ecological Risk Assessment PFAS Food-Web Based Model

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PFAS (per- and polyfluoroalkyl substances) are a large group of environmentally persistent organic pollutants with diverse physical and chemical characteristics. This group of chemicals has recently drawn great concern due to their ubiquitous presence in aquatic environments and the potential hazard they present to aquatic organisms. While these chemicals have important consumer and industrial applications due to their unique properties of heat, grease, oil, and water resistance, these same properties also make them very stable in the environment and difficult to biodegrade. Moreover, due to their ability to bioaccumulate and relatively high-water solubility PFAS can easily partition from their primary sources into aquatic ecosystems and can cause exposures in aquatic life. Determination of bioaccumulation through tissue sample analysis can be expensive and hence models that link toxicity, bioaccumulation, and trophic transfer are needed to better understand the ecological hazards associated with PFAS. In the past year, an Excel-based, food-web based ecological risk assessment model that enables risk assessors to predict PFAS exposures to common and threatened and endangered species in aquatic and terrestrial habitats has been developed. This model allows the user to focus on concentrations of PFAS in abiotic media. The model then applies bioaccumulation metrics to the PFAS measurements in abiotic media to estimate PFAS concentrations at different trophic levels. While the food-web based model for PFAS provides the users with a very convenient tool to make risk estimates, it employs a point-estimate approach and does not incorporate uncertainty and variability into the risk estimate. In contrast, Probabilistic Risk Assessment (PRA) provides a way to characterize uncertainty and variability, enabling decision makers to better assess uncertainty in relation to its effect on decisions. Thus, the goal of this research is to further develop the model from a simple point-estimate format into a probabilistic model using Monte Carlo simulation. In our research, we would present and demonstrate the use of probability distributions for significant input parameters developed based on available qualitative and quantitative data in our PRA model. Such a model enhancement will allow a better characterization of risk and contribute to the overall goal of improving site-specific ecological risk assessments and the development of risk-based screening thresholds for PFAS.

05.06.13 Analysis of Perfluoroalkyl and Polyfluoroalkyl Substances in Water Using UHPLC With a Triple Quadrupole Mass Spectrometer Comparing EPA Methods 537.1 and 8327

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Per- and polyfluoroalkyl substances (PFAS) are a group of man-made chemicals that have been used in a wide variety of industries around the world since the 1940s. This includes equipment used to package and process foodstuffs, commercial household products like nonstick cookware and cleaning products, and industrial goods such as automotive lubricants and electronics, among numerous of other applications. Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) are the two most extensively produced and studied of these chemicals. This class of chemicals are incredibly stable so they are persistent and accumulative in the environment and biological systems. In addition, these compounds were originally considered biologically inert but recent studies have indicated toxicity to both humans and wildlife. Growing health concerns regarding PFASs and their prevalence in consumer goods and the environment indicates a critical need to simply and reliably execute standardized monitoring methods from the EPA and other regulatory bodies on commercially available instrumentation. EPA 537.1 is currently one of the standard methods for analysis of PFASs in drinking water which requires a solid phase extraction (SPE) sample preparation to extract and concentrate the PFAS compounds in the sample in order to measure PFAS compounds the low parts per trillion. EPA Method 8327 is used for the analysis of PFAS compounds in drinking water, ground water and waste water by a direct injection method eliminating the laborious sample preparation required in EPA Method 537.1. This presentation will focus on using the PerkinElmer QSight LC-MS/MS to analyze water samples by both EPA Methods 537.1 and 8327, including a validation study of EPA 537.1.

05.06.15 Evaluation of PFAS in Great Lakes Tributaries and Potential for Biological Effects Using Risk-Based Screening Techniques and Passive Samplers

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Per- and polyfluoroalkyl substances (PFAS) were measured in Polar Organic Chemical Integrated Samplers (POCIS) that were deployed for about 30 days at 20 Great Lakes tributaries (60 sites) in 2018. Sampling sites were selected to represent a range of land uses from urban to agricultural, forested, and wetland influenced. Although several sites were downstream from known sources (airports and wastewater treatment effluent), most sites represented ambient conditions rather than PFAS "hot spots". Of 32 targeted PFAS, 22 were detected, and 17 provided data adequate to estimate water concentrations using available POCIS sampling rates. Between four and eighteen compounds were present at each site (four to fifteen compounds with sampling rates). Site-level concentration sums (for the 17 compounds) ranged from 3.3 ng/L to 283 ng/L, with the exception of one site downstream from an airport that summed to 467 ng/L. Chemicals with the greatest ranked median concentrations were PFOA > PFHxS > PFOS > PFHxA > PFHpA > PFBS. Upstream to downstream comparisons of relative PFAS concentration profiles in several watersheds suggested different source contributions. Concentrations of potential concern (i.e., effects benchmarks for aquatic biota) were derived using bioactivity information from high-throughput screening results, and toxicity data from the peer-reviewed literature. The resulting benchmarks will facilitate a screening-level prioritization of PFAS chemicals, a determination of which sites might be affected, and the relative possibility of biological effects at these sites. *The contents of this abstract neither constitute, nor necessarily reflect, official USEPA policy.*

05.06.16 PFAS Investigation and Risk Assessment at a Flood Control System in Minnesota

C. Archer, AECOM / Environment; H. Loso, AECOM / Environmental Risk Assessment; H.K. Patterson, Dauphin Island Sea Lab / Environmental Risk Assessment; C. Puopolo, AECOM; M. Welsch, AECOM / Environmental Risk Assessment; H. Temme, University of Minnesota / Civil, Environmental, and Geo- Engineering; A. Gorski, AECOM / Environmental Science

A PFAS investigation and risk assessment is being conducted on behalf of the Minnesota Pollution Control Agency at a flood control system in Minnesota that has been impacted by historical releases from industrial disposal sites. The flood control system directs water flow along 14 miles of a diverse system of surface waters in the Tri-Lakes area, including creeks, wetlands, ponds, lakes and rivers. The investigation characterized the nature and extent of PFAS in groundwater and surface water bodies, as well as evaluated the interaction of surface water with shallow groundwater and the interconnected system of bedrock aquifers used for drinking water. A screening ecological risk assessment identified potential risk to birds, mammals, fish, and invertebrates from exposure to surface water and/or sediment, and potentially from foam exposure. PFAS-containing foam is intermittently present and has PFOS concentrations averaging 10,000 times higher than in surrounding surface water.

Additional PFAS sampling was conducted to support a baseline ecological risk assessment (BERA), including collection of crayfish, snails, amphibians, and fish from multiple exposure areas. Co-located surface water, sediment, and porewater data were also collected. The BERA evaluated potential risks for a variety of aquatic and semi-aquatic ecological receptors for more than 20 detected PFAS. Minimal risks to lower trophic level receptors were identified from direct exposures to abiotic media. However, food web modeling with a combination of measured (site tissues) and estimated (literature-based bioaccumulation factors) plant and prey data identified the potential for adverse impacts to piscivorous and herbivorous receptors due to PFAS bioaccumulation. Future work may include data collection to refine the evaluation of risks to herbivores. The findings of the BERA will allow for completion of the conceptual site model and will support remedial decision-making to address PFAS impacts. The presentation will characterize the potential for ecological risks in different exposure areas and will include graphical representations of the various biotic data sets that were evaluated for patterns and trends with regards to the accumulation of specific PFAS in the benthic and aquatic organisms, as well as the abiotic media in which they are in contact. Key uncertainties in the evaluation will be presented.

05.06.18 Zebrafish Toxicity Thresholds for PFOS and Implications for the Protection of Aquatic Life

J. Arblaster, J.M. Conder, Geosyntec Consultants

Multiple environmental agencies have promulgated perfluorooctane sulfonate (PFOS) criteria for the protection of aquatic life. In prior work, we have noted that a single threshold value (0.6 µg/L) for zebrafish (*Dario rerio*) exerts a strong influence on freshwater species protection levels developed by the Environmental Protection Authority of Victoria, Australia. The study from which the 0.6 µg/L value was derived was limited in scope, featuring only 3 PFOS dose levels and only 2 replicates per dose. The value was also more than 10X less than the next-lowest toxicity value in the criteria-derivation dataset and led to a 99% protection level of 0.00023 µg/L, more than 1000X lower than screening values put forth by other regulatory agencies. Given the potential influence of this study result on PFOS aquatic life criteria, the US Army Corps of Engineers Environmental Research & Development Center and the Sediment Management Work Group (with oversight by the US Environmental Protection Agency) partnered to repeat the evaluation of the effects of PFOS to multiple generations of zebrafish with a more robust experimental design. Based on results with the parent generation (lethality, length, and body mass data measured 60, 90, and 180 days post fertilization (dpf) and the total number of viable eggs produced over the last 8 weeks of exposure), the strongest evidence of dose-dependent, potential ecologically significant effects was noted for the 100 µg/L level (highest dose level evaluated). This level indicated minor, but not statistically significant, 14% lethality (relative to controls) at 180 dpf and a statistically significant 21% effect on body mass at 60 dpf (but not 90 or 180 dpf). Minor (3-4%) statistically significant effects on length in male fish (but not female fish) were noted for the 20 and 100 µg/L dose levels, although this level of response does not reflect ecologically significant effects. Importantly, reproduction was unaffected at all exposure levels. While the study is on-going, preliminary results indicate the threshold for ecologically significant effects on zebrafish survival, growth, and reproduction is 100 µg/L or higher, and not 0.6 µg/L. The 99% species protection level calculated using this potential threshold value of 100 µg/L would result in a protection value of 1.3 µg/L, as opposed to the current protection value of 0.00023 µg/L. Additional results from the study will be forthcoming.

05.06.19 Toxicity of PFOS and PFOA to Four Standard Marine Species

N. Hayman, G. Rosen, M. Colvin, NIWC Pacific (Naval Information Warfare Center Pacific) / Energy and Environmental Sustainability; J.M. Conder, J. Arblaster, Geosyntec Consultants, Inc.

Per- and poly-fluoroalkyl substances (PFAS) are emerging contaminants that are coming under increasing scrutiny. Currently, there is a 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 perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) to four standard marine laboratory toxicity testing species, encompassing five endpoints, were evaluated: 1) 96-h embryo-larval normal development for the purple sea urchin (*Strongylocentrotus purpuratus*); 2) 48-h embryo-larval normal development and normal survival for the Mediterranean mussel (*Mytilus galloprovincialis*); 3) 96-h survival of opossum shrimp (*Americamysis bahia*); and 4) 24-h light output for the bioluminescent dinoflagellate *Pyrocystis lunula*. All species were tested using standard United States Environmental Protection Agency and/or American Society for Testing and Materials (ASTM) International protocols. For both PFOS and PFOA, the order of species sensitivity, starting with the most sensitive, was *M. galloprovincialis, S. purpuratus, P. lunula*, and *A. bahia*. The range of median lethal or median effect concentrations for PFOS (1.1 – 5.1 mg /L) and PFOA (10 – 24 mg/L) are comparable to the relatively few toxicity effect values available for marine species. In addition to providing effects data for PFOA and PFOS, this study indicates these species and endpoints are sensitive to PFAS such that their use will be appropriate for deriving toxicity data with other PFAS for marine ecological risk assessment.

05.06.20 Developing Site-Specific Uptake Factor Relationships for a PFAS Investigation and Risk Assessment at a Flood Control System in Minnesota

C. Archer, AECOM / Environment; H. Loso, AECOM / Environmental Risk Assessment; H.K. Patterson, Dauphin Island Sea Lab / Environmental Risk Assessment; C. Puopolo, AECOM; M. Welsch, AECOM / Environmental Risk Assessment; A. Gorski, AECOM / Environmental Science; H. Temme, University of Minnesota / Civil, Environmental, and Geo- Engineering

On behalf of the Minnesota Pollution Control Agency, a PFAS investigation and risk assessment is being conducted at a flood control system in Minnesota that has been impacted by historical releases from industrial disposal sites. The flood control system directs water flow along 14 miles of a diverse system of surface waters in the Tri-Lakes area, including creeks, wetlands, ponds, lakes and rivers. One objective of the PFAS investigation was to collect tissue samples of invertebrates, amphibians, and fish to provide a better understanding of PFAS concentrations in biological tissues at the site and for use in food web modeling to evaluate potential risk for wildlife. Tissue samples were collected in most but not all waterways included in the investigation, and in the absence of waterway-specific tissue data, crayfish and fish concentrations were estimated using uptake factors derived from paired abiotic and tissue samples collected in nearby waterways. Although site-specific uptake factors are more accurate than literature values, the capacity for bioaccumulation is highly dependent upon site conditions as indicated by the variability of abiotic (e.g., sediment, surface water, and porewater) and tissue concentrations among waterways. This presentation will discuss the derivation of PFAS uptake factor relationships for this investigation and the implications of the site-specific uptake factors to understanding the potential for bioaccumulation in upper trophic level wildlife in a food web model. Key uncertainties of inferring uptake of PFAS into biological tissues from different media types (surface water and sediment) and among waterbodies with different PFAS sources and dominant PFAS analytes will also be presented.

05.06.21 A Comparative Analyses of the Worldwide Biomagnification of Per- and Polyfluoroalkyl Substances (PFAS) by the Trophic Magnification Factor (TMF) Perspective

D. Miranda, University of Notre Dame / Biology; G. Lamberti, University of Notre Dame; A. Zachritz, Nova Consulting & Engineering; G.N. Peaslee, University of Notre Dame / Department of Physics

The biomagnifying characteristics of per- and polyfluoroalkyl substances (PFAS) have established several of them on the Stockholm Convention list of the Persistent Organic Pollutants (POPs). Although the Trophic

Magnification Factor (TMF) has been recognized as the most reliable tool for assessing the biomagnification of organic contaminants, peer-reviewed studies reporting TMF are few and with limited geographical distribution. The present work compiles the published literature on TMF for several specific PFAS to access how biomagnification varies in aquatic systems worldwide through a systematic review study. We found 20 published studies evaluating the biomagnification of PFAS with TMF generated through the linear regression between the individual log-PFAS concentration and the δ^{15}N-based trophic position of each organism in the food chains. Our survey of the results shows biomagnification (i.e., TMF>1) of perfluorooctane sulfonate (PFOS) in all cases, with TMF ranging from 1.3 in Hong Kong, China to 17 in Hudson Bay, Canada. The studies analyzed between 1-16 individual PFAS, with PFOS and C_8-C_{14} carboxylic acids the most prevalent PFAS analyzed. Some studies also evaluated PFOS precursors (PreFOS), such as *N*-ethyl perfluorooctane sulfonamide (EtFOSA), perfluorooctane sulfonamide (FOSA), and 6:2 chlorinated polyfluorinated ether sulfonate (F-53B), and found different TMFs contrasts in the biomagnification potential for these PreFOS. Most studies (n = 13) were published after 2015 and are mainly concentrated in China (n=8), Europe (n=6), North America (n=4), and only one study in South America (n=1), and Antarctica (n=1). Generally, the TMFs were higher in the location where a combination of higher trophic levels and a significant contribution of PFAS sources were found intertwined, as in Hudson Bay. However, high TMF for the most analyzed compound, PFOS (i.e., 3–9), was also seen in hotspot sites for PFAS contamination, even with smaller food chains. Due to the concerns regarding its high toxicity, new studies are investigating not only linear PFOS but other different isomers, finding bioaccumulation through the food chain for all PFOS species. This review highlighted that even though the biomagnification of some legacy compounds is well established, the mechanisms driving the specific trophic magnification of PFAS are still poorly studied. We highlight that more studies should be done to better assess the differences in the TMFs for PFAS worldwide.

05.06.22 Critical Review of Frameworks for Screening and Risk Management of Chemicals and Advanced Material

S. Thakali, AECOM / Remediation/Risk; B. Ruffle, AECOM / Environment; D.W. Moore, U.S. Army Corps of Engineers / Engineer Research & Development Center (ERDC); A.D. McQueen, U.S. Army Corps of Engineers / ERDC/EL; D. Edwards, ExxonMobil

Regulatory programs and frameworks developed for assessment of the safety and management of the risks associated with chemicals and/or materials have evolved over the last half-century. However, identification of new and emerging environmental contaminants continues, which suggests a need for review of current approaches and strategies. Twelve existing frameworks relating to assessment and management of chemical/material risk were reviewed to identify potential process improvements for facilitating early identification of potentially problematic substances (or properties) and better inform chemical/material risk management strategies (e.g. prohibition, restricted use and/or selection of safer alternatives). The frameworks selected for review represent a broad spectrum of regional, national, and international authorities and purposes, including pre-production evaluation of new substances, classification and hazard communication, identification of persistent pollutants, and identification of safer alternatives. Elements common to the frameworks were identified (e.g., tiered, and iterative construct, use of weight-of-evidence in decision-making, application of risk assessment principles), as well as features unique to select frameworks (e.g., lifecycle analysis, application of multi-criteria decision analysis, socioeconomic considerations). A comparative evaluation was performed; and potential new strategies and approaches were identified to inform recommendations for process improvement. These recommendations include development and application of validated analytical procedures, use of new approach methodologies, and increasing data transparency, accessibility, and usability to facilitate future high throughput assessments (e.g., machine learning and artificial intelligence). Additionally, incorporation of monitoring and adaptive management post-registration could provide an important stop-gap for minimizing and/or reducing future risks. This presentation will discuss commonalities and unique elements among the frameworks reviewed and present recommendations for areas of future improvements to enhance more timely identification of problematic substances.

05.06.23 Setting Thresholds for Forever Chemicals- Derivation of Environmental Quality Guidelines for Perfluorooctanoic Acid (PFOA)

T. El-Fityani, J. Cermak, D. Spry, Environment and Climate Change Canada / National Guidelines and Standards Office, Science and Risk Assessment Directorate

Perfluorooctanoic acid (PFOA) is a man-made substance that falls under the broader class of chemicals known as per- and polyfluoroalkyl substances (PFASs). These substances have recently been referred to as "Forever Chemicals", owing to their persistence and their tendency to accumulate in organisms. The Government of Canada previously concluded that PFOA, its salts and its precursors are toxic to the environment. As such, tools are needed to help assess and manage the risks of PFOA to environmental receptors in the ambient environment and at contaminated sites. One such set of tools are environmental quality guidelines, which are science-based benchmarks for environmental protection based on hazard. Monitoring data can be compared against the guideline values to inform site screening, risk assessment and/or risk management. Environment and Climate Change Canada is currently deriving PFOA guidelines for environmental protection for surface water, groundwater, soil, sediment, wildlife tissue, and bird egg, as data permit. These guidelines represent concentration thresholds below which there is low likelihood of adverse impacts from PFOA on ecological organisms or, in the case of soil, below which important ecological functions of the land will be maintained. The derivation process will be discussed and available draft guidelines will be presented.

05.06.24 Wastewater Treatment Plant, Air Pollution Profiles, Odor Annoyance, and its Effects on the Human Health

V. Clower, Georgia Southern University / Jiann-Ping Hsu College of Public Health; C. Adeoye, Georgia Southern University / Environmental Health Sciences

Several environmental contaminants, including malodors, ammonia, volatile organic compounds (VOCs), and fine particulate matter (PM), are released into the atmosphere during the process of wastewater treatment by wastewater treatment plants (WWTPs). The odors are primarily due to release of ammonia, hydrogen sulfide, and organic compounds of sulfur and nitrogen. These contaminants may pose risk to humans living close to WWTPs and plant employees. The objective of this research was to investigate the air pollution profiles and the exposure level of residents nearby the wastewater treatment plant to particulate matter, total volatile organic compounds, and to use a health survey to understand the relationship between unpleasant WWTP odor exposure and the health of individuals. There is limited data available on the level of these pollutants at several distances from the point sources. Availability of data on contaminant levels close to these point sources can help inform decision making for the public especially those living close to these sites. The proposed study will address these knowledge gaps. We formulated a standardized questionnaire to help us understand: the relationship between unpleasant WWTP odor exposure and the health of an individual; and the relationship between the seasons and the unpleasant WWTP odor produced from the WWTP. The number and mass concentration PMs of sizes (1.0, 2.5, and 10.0 µm), TVOCs, and ammonia data were collected by using CEM DT-9881, DustTrak™ II Aerosol Monitor 8532, VOC08 meter, and an ammonia detector, respectively. Airborne PM1, PM2.5, and PM10 mass concentrations were measured 6 times at each location with a DustTrak™ Aerosol Monitor 8532 (TSI Inc., Shoreview, MN). Two residential apartments close to the Waste-Water Treatment Plant were the nearby communities selected to carry out the sampling and health survey. The sampling sites were grouped into three categories based on distance:

Group 1(150m), Group 2(250m), and Group 3(350m) distances from the WWTP. Sampling was carried out three times in a day: 9am in the morning; 2pm in the afternoon; and 5pm in the evening. This was done three times in a week for eleven weeks. We hypothesized that the level of the air pollution profiles will be higher at the communities close to WWTP. In this study, the number and mass concentrations of PM1, PM2.5, and PM10, TVOC, and ammonia concentrations were measured outdoor in residential communities near wastewater treatment plant. Group 1 had the highest level of TVOCs, and group 3 had the lowest level of TVOCs. The concentrations of PMs (1.0, 2.5, and 10.0 µm) showed no trend across the mornings, afternoons, and evenings of the data collection. Eye, nose, and throat irritation are the symptoms the participant noticed during periods of a higher concentration of odors coming from the WWTP. The mean concentration of PM1, PM2.5, and PM10 throughout our study were in the range of 0-14 µg m-3, 1-17 µg m-3, and 2-31 µg m-3, respectively. Individuals of the questionnaire considered the Statesboro wastewater treatment plant to be the source of the malodor and yet to perceive malodor from another source.

05.07 Incorporating Climate Change Predictions into Human Health and Ecological Risk Assessments

05.07.01 The Impact of Global Climate Change on the Estimation of Ecological Risk and the New Seven Conclusions

W.G. Landis, Western Washington University / Institute of Environmental Toxicology and Chemistry

In 2011 a Pellston workshop was held to evaluate the impact of global climate change (GCC) would have on the fields of environmental toxicology and ecological risk assessment. The papers from this workshop, published in Environmental Toxicology and Chemistry in 2013, and remain the key references on the topic. This presentation focuses on current ecological risk assessments and the realizations in how climate change has to be taken into account. In the spirit of the original publication here is a list of seven conclusions. 1) The past is not the future, our datasets since the 1940s have been taken during the forcing of anthropogenic climate change. 2) Over the last decades measurement technologies have changed dramatically meanwhile the historic data and supporting metadata get lost. So it is now difficult to reconstruct the past trajectories of both GCC and contaminant loadings. 3) Reference conditions did not and will not exist except as design goals. 4) GCC is one of multiple stressors from chemicals to nonindigenous species and we should expect close interactions. 5) Changing to a hydrogen or other economy will bring new stressors with unknown exposure-response relationships and the associated uncertainties 6) There will be Black Swans, the future is uncertain and assumptions should be revisited on a regular schedule. 7) Finally, I suggest developing tools appropriate to the questions, not adjusting the questions to be amenable to those old tools. The upcoming questions will be hard and we need to develop the risk assessment techniques to address them.

05.07.02 Do Current Modeling Scenarios Address Changes in Climate Patterns?

A. Ritter, Waterborne Environmental, Inc. / Exposure Modeling; J.M. Jackson, A. Schmolke, Waterborne Environmental, Inc. / Effects Assessment; G. Hoogeweg, Waterborne Environmental, Inc. / Exposure Modeling; K. Marincic, Waterborne Environmental Inc. / Field Services; J. Eble, Eble Group

Standard regulatory ecological risk assessments in the US and Europe rely on historical weather data to predict pesticide concentrations in groundwater and surface water. With climate change, it is predicted that temperatures will increase and rainfall patterns will shift. It is also expected that many areas will see a change in the amount of rainfall and a change in rainfall intensity. Current climate models essentially predict that arid regions will get drier and wet areas will get wetter. The effects of climate change on pesticide risk assessments are multiple. With higher temperatures, an increase in degradation of pesticides is expected and will reduce predicted concentrations in groundwater and surface water. However, increased rainfall amounts and/or intensity may result in increased leaching and runoff, leading to higher concentrations of pesticides in groundwater or surface water. In arid areas, due to a decrease in rainfall, additional irrigation may be required, the consequences of which are still poorly understood. Currently no modeling scenarios are available to predict these effects. We have developed an approach that enables us to update existing standard weather files from USEPA, PMRA, EFSA, etc., with climate change data to incorporate predicted changes in rainfall and temperature for any standard scenario (met file), agricultural field or watershed. The resulting data are daily weather files that account for increased temperature, changes in rainfall and rainfall intensity as predicted by the climate change models and the different climate change scenarios. In this presentation we will show the effects of climate change on a risk assessment in an arid region and a wet region for standard pesticide substances with different environmental fate properties. The results will demonstrate the potential effects of climate change on predicted environmental concentrations in groundwater and surface water.

05.07.03 A Bayesian Network Approach to Probabilistic Risk Assessment of Pesticides Under Current and Future Agricultural and Climate Scenarios

S. Mentzel, M. Grung, NIVA / Ecotoxicology and Risk Assessment; R. Holten, M. Stenrød, Norwegian Institute of Bioeconomy Research NIBIO / Department of Pesticides and Natural Products Chemistry; K. Tollefsen, J. Moe, Norwegian Institute for Water Research (NIVA) / Section of Ecotoxicology and Risk Assessment

Future weather patterns are expected to result in increased precipitation and temperature in Northern Europe. These changes can potentially cause increased occurrences of plant disease and pests which may require alterations in agricultural practice, such as the crop types and application patterns of pesticides. We use a Bayesian network to explore a probabilistic risk assessment approach, to better account for variabilities and magnitudes of pesticide exposure to aquatic ecosystems. As Bayesian networks can link input and output variables from process-based models and other information sources, they can serve as meta-models. In this study, we use a pesticide fate and transport model (WISPE) with specific environmental factors such as soil and site parameters together with chemical properties of pesticides and climate scenarios that are representative of a Norwegian study area. The predicted pesticide exposure of the study area is integrated with effect distribution derived from toxicity tests in the Bayesian network model, to predict the risk to the surrounding aquatic ecosystems (streams). This Bayesian network model enables incorporation of future climate projections into ecological risk assessment.

05.07.04 The Use of Quantitative, Probabilistic Ecological Risk Assessment to the Release and Application of Synthetic Biology for Adaptive Environmental Management

W.G. Landis, Western Washington University / Institute of Environmental Toxicology and Chemistry

The 2016 NASEM report *Gene Drives on the Horizon* outlined a risk assessment framework and specified the requirements for the ecological risk assessment of gene drive applications for release to the environment. Much has been said regarding such applications but as of 2021, no risk assessment has been conducted that meets these specifications. Recently, our research group has been exploring possible approaches for two case studies. One is exploring the ability to gene drives to reduce a population of a nonindigenous mouse on an island. The second is the control of disease carrying mosquitos in the Ponce region of Puerto Rico. The risk estimation method is a derivation of the Bayesian network relative risk model with the integration of the Marshall et al (2017) approach for estimating gene drive propagation through a population. Adaptive management is characterized using the approach of Landis et al 2017. We will use these two examples to compare to the NASEM guidance and current approaches. Finally we point to the development needs to actualize gene drive risk assessment and its application to the modification of ecological structures, whether it is the control of disease, the elimination of nonindigenous species or the building of coral reefs resistant to climate change.

05.07.05 Overview of a Proposed 2022 SETAC Pellston-Style Workshop on Integrating Ecological Risk Assessment With Climate Change Predictions

R.G. Stahl, DuPont (retired); K. Brix, EcoTox LLC; J. Moe, Norwegian Institute for Water Research (NIVA) / Section of Ecotoxicology and Risk Assessment; A. Boxall, University of York / Department of Environment and Geography; W.G. Landis, Western Washington University / Institute of Environmental Toxicology and Chemistry; J.L. Stauber, CSIRO / CSIRO Land and Water

In 2011 a SETAC Pellston Workshop was held on Global Climate Change. Seven publications resulted from the workshop, all published in our SETAC journal *Environmental Toxicology and Chemistry*. One of the major issues we were unable to address at that time was how to integrate climate change predictions into ecological risk assessments. This was due, in large part, because the models that underpinned climate change predictions were focused on spatial scales much larger than would be useful for ecological risk assessments. Since the workshop in 2011, climate change modelers have been able to downscale their models and predictions to spatial scales more compatible with those needed for ecological risk assessments. Meanwhile, eco-toxicologists and risk assessors have become more familiar climate model projections and their associated uncertainty. In our presentation we will provide an overview of the charge questions we plan to address, the focus of the manuscripts we plan to develop and publish, as well as invite the audience to comment on the proposed workshop so that we may consider that input for improving our approach.

05.08 Integrated Approaches to Identify and Quantify Human and Ecological Risk

05.08.01 Using Scalar Approaches for Integrating Human Health and Ecological Risk Assessments

C. Menzie, M.W. Kierski, Exponent / Ecological and Biological Services

Scalar approaches translated into simple visual presentation provide a means of integrated communication of human health and ecological risks. Currently these risks are usually presented separately using a variety of absolute and relative metrics. Difficulty arises on the part of decision makers on how to assimilate such disparate information and understand the merits of proposed alternative actions. We show how myriad health and ecological risks can be presented together using a relative approach anchored to either quantitative (absolute risk) or relative risk scales. The visual representations are underpinned by widely used and familiar endpoint-specific health and environmental metrics. These are not added but presented simultaneously using a common scalar framework with color coding indicating typical determinations on risk acceptability or degree of risk. This allows easy visual comparisons of alternatives along with measures of risk reduction and efficacy. The paper provides a table of metrics amenable to this form of analysis. Familiar examples include cancer risks for humans and the Index of Biological Integrity (IBI) for stream biota. Ecological risks can be evaluated in terms of classical receptor endpoints or in terms of ecosystem services. An example of application to decisions regarding water quality in coastal systems - Boston Harbor and Chesapeake Bay - will be provided.

05.08.03 Assessing Ecological Effects of Anthropogenic Marine Debris Removals on Coral Reef Communities in the Florida Keys

T.G. Payton, Clemson University / Department of Biological Sciences

The Florida Keys has undergone severe loss of hard coral reef habitat due to anthropogenic stressors and climate change. This loss of habitat has resulted in the shifting of species community structures from structurally complex hard coral cover to systems more dominant in soft coral and macroalgae. It is hypothesized that increases in debris may increase structural complexity on these shifting reefs. Little research has been conducted on biota community responses to debris as artificial habitat, however; studies showing behavioral responses to artificial reefs and habitat loss may be mimicked by debris and influenced by its removal.The Florida Keys National Marine Sanctuary has developed the Goal Clean Seas program to train local dive shops to become Blue Star Operators in an effort to aid in the removal of increased debris pressure following Hurricane Irma in 2017. However, as organisms use this debris to a greater extent there is concern for what species are most impacted by the removal and displacement of artificial habitat. To assess ecological risk of common marine debris clean up events, organisms removed as a result of debris removals from Blue Star Operators in the Florida Keys were recorded, weighed, and categorized by functional group. Functional groups of concern and biomass removed are used to create a risk assessment of clean up events in an effort to inform better management practices regarding debris removal.

05.08.04 Comparison of Fish Fillet Sample Preparation Methods for Superfund Human Health Risk Assessments

A. DeBofsky, S. Kim, U.S. Environmental Protection Agency / Superfund and Emergency Management Division; D.J. Getty, Leidos; M.S. Greenberg, U.S. Environmental Protection Agency / Superfund HQ; C.G. Nace, U.S. Environmental Protection Agency / Superfund and Emergency Management Division

Contaminant concentrations in fish are crucial for evaluating the risk to public health and developing remediation goals at Superfund sites with contaminated surface water and sediments. The US Environmental Protection Agency (EPA) Region 2 and New York State Department of Environmental Conservation (NYSDEC) support two different methods for preparing fish fillet samples for tissue residue analysis. Current EPA methods of fish sampling for use in human health risk assessments require that fish are filleted, and no rib bone is to be included; however, NYSDEC requires that the fish fillets include the rib bones. Information on how each fish filleting method affects the measurement of contaminant concentrations is currently limited, thereby adding uncertainty in comparisons of historic and/or current samples that might have been collected with different filleting techniques. This study primarily determined the variability in filleting strategies and examined how intra- and inter-laboratory variability can influence contaminant concentration results. To accomplish these objectives, 12 striped bass and 13 bluefish were collected from multiple locations within Jamaica Bay, NY. Fillets were excised from each side of the fish; one was prepared with the rib included while the other did not contain the rib. Fish tissues samples were sent to two laboratories for the analysis of polychlorinated biphenyls (PCBs), mercury (Hg), and percent (%) lipids. This study revealed that lipid content was 2% and 30% greater in rib-in samples than rib-out samples for bluefish and striped bass, respectively. Additionally, lipid-normalized concentrations of PCBs (LPCBs) were 8% and 12% greater in samples with the ribs in than without ribs in bluefish and striped bass, respectively, and sample preparation method did not affect the measured concentrations of mercury. Intra-laboratory results of replicate measurements showed no statistical difference between replicates for any of the parameters. Concentrations of lipids and LPCBs had coefficients of variance as high as 53% between lab analyses; however, a low sample size analyzed in both labs (n=3) limits the results. Therefore, while the data suggest that inter-lab variation may outweigh any differences present from filleting technique, this finding needs to be explored in more detail. Ultimately, this study provides support for the direct comparison between EPA and NYSDEC fillet concentrations for risk assessment purposes.

05.08.05 Using Version 2 of the Ecological Risk Classification of Organic Substances Approach to Identify Chemicals of Global Concern

D. Bakker, K. Ratneswaran, D. Lee, Environment and Climate Change Canada; M.A. Bonnell, Environment and Climate Change Canada / Ecological Assessment Division

Since 2016, Environment and Climate Change Canada has used a multi-descriptor weight of evidence approach known as the Ecological Risk Classification of Organic Substances (ERC) to prioritize organic substances for further evaluation and risk assessment under Canada's Chemicals Management Plan. The second version of the approach (ERC2) was developed in 2018 to incorporate confidence and severity scoring. One of the benefits of these approaches is that they are risk-based, allowing for the evaluation of both hazard and exposure potential. The ERC approach incorporates descriptors to identify highly potent chemicals at different spatial and temporal scales of exposure with one of the goals being to target substances that have the potential to affect the planetary boundary. The concept of the planetary boundary for chemical pollution suggests that the Earth has a finite assimilative capacity for chemical pollution. ERC2 builds on the work of other scientists who have attempted to define specific criteria for this boundary. Chemicals that may pose threats to the planetary boundary for pollution (along with their transformation products) are typically persistent and mobile substances that have a hazard potential capable of causing permanent genetic damage in exposed organisms, and which may also cause epigenetic changes in subsequent generations. These effects of concern are irreversible or poorly reversible, or result in a regime shift to a new stable ecological state. Effects of these chemicals distribute widely across populations and are independent of global or local concentrations. Since exposure at very low concentrations can result in adverse effects, concentrations in the far field – usually diluted compared to near field or local concentrations – become highly relevant. ERC2 prioritizes substances of this type as "chemicals of global concern" (CGC) where the risk can scale up to the far field. In this poster, a case study with approximately 60 substances that are used as flame retardants will be presented to show how ERC2's rule-based logic classifies substances as CGCs. Rules for classifying hazard (e.g., chemical reactivity, genotoxicity, receptor-mediated toxicity) as well as exposure (e.g., response time, mobility, food web exposure) and risk along with associated confidence will be presented.

05.08.06 Investigation of Human Exposure Sources and Estimation of Exposure Reduction Effects by Environmental Remediation Using IEUBK Model in Lead-Contaminated Area

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The US Environmental Protection Agency (USEPA) Integrated Exposure Uptake Biokinetic (IEUBK) model is generally used to estimate blood lead levels (BLL) in children from environmental lead (Pb) levels. In the Kabwe mining town, Zambia, extremely high Pb levels have been reported. However, a comprehensive assessment of Pb exposure through foodstuff and water has never been performed, resulting in a lack of knowledge of major sources of human exposure. Although various environmental remediation techniques have been suggested, the extent to which these measures reduce BLL is unknown. This study aimed to investigate major sources of human exposure in this area and to estimate the effect of remediation on the BLL reduction through the developmental use of the IEUBK model.Twenty-one sampling points in 13 areas were set up within a range of approximately 10 km from the mine to collect samples of soil, house dust, drinking water, maize, and vegetables. The samples were subjected to metal analysis, and the BLL was estimated for each area and age category using the IEUBK model. Then, the IEUBK model was again used to simulate the change of BLL with decreasing soil Pb concentration.Soil Pb levels ranged from 6.7 to 15,000 mg/kg with a mean of 1,900 mg/kg while the USEPA's reference level is 400 mg/kg. Lead levels in all the studied leafy vegetables and onions exceeded the reference level of 0.3 and 0.1 mg/kg, set by FAO/WHO, respectively. In contrast, the mean water Pb level was 0.5 µg/L, which is much lower than the standard level of 5 µg/L, set by the European Union. The estimated mean BLL in the most polluted area ranged from 26.9 to 32.4 µg/dL by age while the observed mean BLL in our previous study in 2017 ranged from 34.0 to 69.4 µg/dL. Estimation for 75% reduction in soil Pb levels showed that the estimated BLL was reduced by only 30% at most although this estimation does not consider the decrease in house dust and food Pb levels that accompanies the decrease in soil Pb level. In contrast, estimation for 75% reduction in food Pb levels reduced the estimated BLL by 69% at most. Our results suggested that major exposure source would differ by contamination level of each area.Ingestion of contaminated food would a major Pb exposure source in relatively low contaminated areas albeit higher contribution of the oral and respiratory exposure to the soil in more polluted areas. The 75% reduction in food Pb levels is considered to reduce BLL by approximately 10-70%.

05.08.07 Toxicity Assessment in Human Population Exposed to Pollutants From Madín Dam Located in the State of Mexico, Mexico

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Madín dam is a reservoir that supplies 2% of the drinking water to the municipalities of Naucalpan de Juárez and Atizapán de Zaragoza; recreational activities are also carried out, such as fishing for various species, including common carp, which is used as food by the residents of the communities of Nuevo Madín. Several groups of contaminants have been detected in this water body, including metals, pharmaceuticals, pesticides and persistent organic pollutants, which have been shown to cause oxidative stress, genotoxicity, cytotoxicity, embryotoxicity and teratogenesis in aquatic organisms. Therefore, toxicological studies have been carried out, and showed that the contaminants present in the water produce oxidative stress, genotoxicity, cytotoxicity, embryotoxicity and teratogenesis in aquatic organisms. The water from this reservoir is purified and distributed to the surrounding populations, so the objective of this work was to evaluate a set of biomarkers to determine the damage induced by exposure to contaminants from the Madín dam on the human population. For this purpose, three groups were studied: the reference group (Z3), which refers to people living in any neighborhood belonging to the Metropolitan Zone of the Valley of Mexico, but unrelated to Madín dam; the Nuevo Madín group (Z1), formed by people living in the vicinity of the dam and receiving water from the reservoir; and the Lomas Verdes group (Z2), which refers to people living in neighborhoods that receive drinking water from the reservoir, but are not located in the vicinity of the dam. Men and women between 18 and 60 years of age, apparently healthy, whose residence time in the colonies of interest was greater than 10 years, were recruited. The assessment of the damage induced by exposure to contaminants was performed by degree of lipoperoxidation and the quantification of advanced protein oxidation products, as well as the activity and gene expression of the antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase. The frequency of micronuclei in peripheral blood and the degree of DNA methylation were also determined. The results showed alterations in the oxidative state, an increase in the frequency of micronuclei and in the degree of DNA methylation in the research subjects living near the Madín dam and in those who receive drinking water from this reservoir, with respect to the research subjects who are not related to this body of water.

05.08.08 Using Scientific Workflows to Evaluate Human and Environmental Impacts From Chemical Exposures

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Holistic evaluation of chemical impacts requires integration of disparate data streams, modelling of interactions among physical and biological systems, and consideration of tradeoffs associated with management decisions. Over the years, environmental health scientists have developed a variety of models that use available data with other mechanistic information to understand how chemicals in products move through the natural and built environment. Each new problem incrementally advances data, models, and understanding. To efficiently address problems of increasing complexity and enable greater insights, more nimble modeling methodologies are required to assess important chemical exposure scenarios and pathways across the chemical/product lifecycle. A scientific workflow is designed to execute a series of data manipulation and computational steps to provide outputs tailored to decision-making contexts. For chemical assessments, scientific workflows are useful tools for efficient and transparent analyses, especially for considering impacts in complex systems. To demonstrate this approach, we use relatively data-rich chemicals as test cases to drive conceptual workflow development including flame retardants and ethoxylated solvents. The goal is to advance the workflows for efficient evaluation of a variety of chemicals and a range of decision contexts. 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.

05.08.09 Comparative In Vitro Toxicity Assessment of Human Pregnancy Exposome Based on Urine Mixtures Using a Quantitative Toxicogenomics-Based Yeast Assay

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Within the goals of NIEHS-funded PROTECT center, one of the objectives is to develop technologies to unravel the relationship between the human pregnancy exposome and adverse pregnancy outcomes. The intrinsic limitations in current individual chemical- or biomarker-based exposome assessment approaches call for a paradigm shift to the comprehensive evaluation of mixture exposure implicating multiple toxicity pathways. Compared to other biological matrices that are commonly used for human exposure assessment, urine is easy to obtain in large volumes and contains both the metabolites and the non-metabolized chemicals, which reflects internal exposome in close proximity. Therefore, developing a relevant urine mixture toxicity assessment tool is in need for individual risk-driven and effect-based exposure monitoring. *In vitro* omics-oriented toxicity evaluation approaches have shown rising potential for cost-effective and feasible screening of mixture toxicity. In this study, a recently established quantitative toxicogenomics-based yeast assay was employed for the comparative toxicity evaluation of the organic extracts of urine samples collected from the households of pregnant women in Puerto Rico. Urinary dilution was corrected based on specific gravity, and glucuronide-conjugated metabolites were hydrolyzed prior to the toxicity analysis. Seventy-six key proteins indicative of DNA stress, general stress, oxidative stress, chemical stress and proteins tress, were selected as cross-species-conservative molecular toxicity biomarkers using GFP-fused yeast-reporters. Exposome-specific toxicity fingerprints and distinct pathway-level perturbations were revealed among different individuals. Quantitative molecular toxicity endpoint Protein Effect Level Index (PELI) was derived, allowing for the comparison of toxicity profiles among samples. A PELI value above the toxicity threshold 1.5 was observed in all urine mixtures. Chemical stress and DNA stress dominated the toxicity effects in 6 and 5 out of 13 samples, respectively. The correlations of the information-rich toxicity data with parallel urine metabolite analysis and adverse pregnancy outcome results are being evaluated. This study reports an innovative toxicogenomics-based approach for the evaluation of human exposome based on urine mixtures, which can provide high-resolution complementary information to other exposure assessments, and can be potentially incorporated into the health risk assessment framework.

05.08.10 Regulation of Cannabis Contaminants in the U.S.: Inconsistencies in Limits, Lack of Relevancy to Agriculture, and Need for Centralized Oversight

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Growing interest in legalizing cannabis in the U.S. has led to disparate approaches towards the necessary safety regulations surrounding this crop at the state level. This inconsistency in the regulation of cannabis agriculture presents a potential challenge to human health and the environment. Here, we reviewed the regulated contaminants in medical and recreational cannabis in 33 states and Washington, D.C. and compiled complete lists from regulatory documents for pesticides, inorganics, solvents, and microbes/mycotoxins. Twenty-eight of the 34 jurisdictions set contaminant limits or action levels, 21 of which created their own contaminant lists. The remaining 7 jurisdictions compiled lists from other sources. These sources included (i) the Herbal Medicine Compendium; (ii) the U.S. EPA 40 CFR Part 180 Tolerances and Exemptions for Pesticide Chemical Residues in Food; and (iii) the pesticides examined by the USDA in its 2010-2011 Pilot Study: Pesticide Residue Testing of Organic Produce. All but one state attempted to categorize contaminants. Yet, many of these contaminants, particularly pesticide-related chemicals, were improperly categorized. Using the U.S. Environmental Protection Agency CompTox Chemistry Dashboard, we generated a comprehensive list of over 700 unique contaminants regulated in the U.S. which included pesticides, solvents, inorganics, and microbes/mycotoxins. We found large variations in regulatory action levels that could not be explained by patterns of use, human health risk, or environmental concerns. For instance, three states applied the same action level to all of their regulated contaminants. These regional variations allow for contaminated products to be sold in certain states with more relaxed action levels that would not pass testing in a different state. Since medical and recreational cannabis consumption is increasing in the U.S. and the number of states with some degree of legal use continues to increase, the lack of federal oversight has led to a scattershot approach to the regulation of contaminants and contaminant levels in this industry. This may either confuse growers or encourage overuse of chemicals, resulting in potential health risks to the consumers as well as adverse effects on the environment.

05.08.11 Environmental Suitability Analysis for Cannabis and Hemp Growth in Arizona

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With the passage of Proposition 207, the emerging cannabis and hemp industry has become a key environmental concern in Arizona. The potential environmental impact of indoor and outdoor growth can be investigated using a geospatial approach to analyze current agricultural practices and their impact on urban and rural areas. Here, we utilize geographic information systems (GIS) and conduct a preliminary analysis for cannabis and hemp growth in Arizona. We compare geographic information concerning water presence and land ownership to documented cannabis and hemp growth sites. Significant patterns are noted for use in further analysis on weighing how important the identified factors are to cannabis and hemp growth. These factors can then inform hotspot analysis to predict which areas are most affected by cannabis and hemp growth due to wastewater runoff, air pollution, and energy use. Our analysis reveals three relevant significant patterns. First, both hemp and cannabis growth are strongly tied to water presence. Second, hemp growth occurs mainly in agricultural designated land but cannabis growth does not. Third, both cannabis and hemp tend to be grown in proximity to moderately developed areas. Further studies will focus on Central Arizona and Phoenix and statistically determine the optimal locations for hemp and cannabis growth. The expected outcome of this study will inform environmental monitoring of air and water pollution as well as targeted surveys of farmers and residential communities. This will provide crucial exposure and risk information to support environmental regulation in Arizona.

05.08.12 Utilizing Zebrafish Bioactivity Screening to Characterize the Risk of the Most Commonly Occurring Chemicals from Global Wristband Personal Passive Sampling Studies

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The development of small, personal passive sampling devices like silicone wristband samplers has revolutionized our ability to assess interindividual variability in personal chemical exposures. This technology gives researchers the ability to compare chemical exposure profiles and identify common exposures within and across populations. A recently published study utilizing wristband samplers identified a group of 14 chemicals detected in extracts from over 50% of samplers deployed across three continents. Of these common chemicals, most are present in consumer products, representing a large percentage of personal exposure across demographics. The high frequency of exposure to these chemicals elicits questions of their potential adverse human health effects. Additionally, the possibility for exposure to mixtures of these compounds is likely, due to their co-occurrence, so the potential for mixtures to induce differential bioactivity warrants further investigation. The zebrafish is an established model for human health-related research and chemical screening. Using early life-stage zebrafish, we assessed the bioactivity of the 14 compounds individually and in combination, assessing 22 morphological and 2 sensitive behavioral endpoints. Of the individual compounds, 10 exhibited bioactivity, but with variable potency based on benchmark concentration which induced a 50% effect (BMC_{50}) relative to control groups for each chemical. Additionally, multiple submixtures of chemicals induced greater bioactivity than any individual compound alone, indicating potential chemical interactions within the mixtures, warranting future mixture interaction work. This study offers exciting possibilities to broadly evaluate and communicate the hazards posed by the most relevant combinations of human chemical exposures by coupling passive chemical sampling with high-throughput toxicity screening in zebrafish.

05.09 Nanoplastics Part II: Effects and Risks

05.09.01 Evaluation of Selected Bio- and Degradable Plastics Weathered in the Marine Environment by Pyrolysis GC/MS

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As concern about the impact of conventional, petroleum-based plastics grows, bioplastics (BP) and degradable plastics (DPs) have emerged as potential sustainable alternatives. But the fate of BPs and DPs in the environment remains unclear and some may breakdown into nano- and micro-plastics forms in a similar manner to petroleum-based plastics. Potential adverse ecological effects of nano- and micro-BPs and DPs are also unknown. If they do form nano- and/or micro-BPs and DPs, methods and approaches will be needed to measure and identify these classes of plastics under environmental conditions. To evaluate methods for identifying and quantifying these new plastics and to elucidate the fate of BPs and DPs in the environment, samples of cellulose acetate (CA), polycaprolactone (PCA), polylactic acid (PLA), polyhydroxybutrate (PHB), polybutylerne sebacate (PBSe) and polybutylerne sebacate co-terephthlate (PBSeT) were selected for assessment and subjected to simulated marine weathering conditions including ultraviolet light exposure, temperature, mechanical abrasion, and seawater. To initiate the investigation, selected plastics were mechanically degraded into macroplastic sizes (i.e., >5 mm) likely to enter the marine environment via improper-disposal practices. Following weathering manipulations, unweathered and weathered BP and DP samples were analyzed by pyrolysis-GC/MS to determine if weathering affected the ability to detect and characterize the plastics in environmental samples. The next set of experiments will assess the fate of the plastics under weathering conditions including the probability of the formation of nano- and microplastics. Findings from this investigation will provide useful information for better understanding the environmental fate of BP and DP in marine waters.

05.09.02 Assessing Polymer Weathering in Aquatic Environmental Systems

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Sunlight has been shown to be an important determinant of risk associated with exposure to polymer residues present in aquatic environments. Energy provided by absorption of solar photons can initiate a wide range of reactions, including direct and indirect photolysis that results in the weathering of polymers. Inputs of trash represent a recurring opportunity for contamination of aquatic and terrestrial environments by polymer-derived compounds. Here we present data based on the simulated environmental weathering of different polymeric nanocomposites, (epoxy, polyamide, polypropylene) filled with organic (multiwalled carbon nanotube, graphene, carbon black) and inorganic (WS_2, SiO_2, Kaolin, Fe_2O_3, Cu-Phthalocyanines) nanomaterials. Sunlight plays a major role in the weathering of these polymers, which results in dramatically different fragmentation to micro- and nano-sized particles. Multiple techniques were employed to extensively evaluate the effects of weathering of the polymers on fragmentation products: ultraviolet-visible spectroscopy (UV-Vis), Fourier transform infrared spectroscopy (FTIR), optical microscopy, contact angle measurements, gravimetric analysis, analytical ultracentrifugation (AUC), transmission electron microscopy (TEM), scanning electron microscopy (SEM) and Raman spectroscopy. This work aimed to elucidate the extent to which weathering protocol (*i.e.* wet vs. dry) and diverse filler characteristics modulate fragment release and polymer matrix degradation. Other studies of weathering of low-density polyethylene- nanosilica composites also were conducted. The polymer was modified by addition of a pro-oxidant to stimulate transformation of the polymer matrix to more biodegradable forms. Polymer degradation was followed by Fourier-transform-infrared spectroscopy (FTIR) and changes in tensile mechanical properties. Addition of the pro-oxidant accelerated deterioration of mechanical strength by an order of magnitude compared to controls. Wavelength studies demonstrated that the short-wavelength component of simulated sunlight was most effective in inducing photodegradation of the matrix with added pro-oxidant. Action spectra based on these wavelength studies were used to evaluate the effects of sunlight over space and time on photodegradation of this polymer.

05.09.03 IRRSM Spectroscopic Identification of Microplastic Contamination of <20u Using Simultaneous Submicron IR + Raman

J. Anderson, Photothermal Spectroscopy Corp / Product Development

Microplastics (MPs) have gained tremendous exposure in recent years, in scientific circles, but also with the broader public through media coverage. Thus far, analytical scientists working on these challenges have turned to traditional chemical analysis techniques, such as Fourier transform infrared (FTIR) microscopy and Raman microscopy, but these techniques have their limitations, especially as interest grows in the small, < 20 µm size fraction, which is known to be the more biologically relevant, but more analytically challenging to measure size fraction. Traditional techniques like FTIR are spectrally rich, but have limited spatial resolution and cannot measure < 20 µm. FTIR, when operating in reflection or transmission mode is also plagued by dispersive scattering artefacts (Mie scattering) which distort spectra and reduces the identification accuracy . Raman, whilst possessing excellent spatial resolution, down to ~1 µm , suffers from autofluorescence issues and limited sensitivity. Here we present a new breakthrough IR spectroscopic approach termed, Optical Photothermal IR (O-PTIR) spectroscopy that essentially combines the best attributes of IR and Raman into a single instrument. O-PTIR, operates in reflection mode (non-contact, far-field), delivers submicron spatial resolution with FTIR transmission-like spectral quality and sensitivity in seconds. Due to the unique system architecture, the system can be extended with Raman to provide for simultaneous IR+Raman microscopy, thereby providing both IR and Raman spectra (same spot, same time, same resolution), from submicron particles (and larger) simultaneously, in one measurement in seconds. This technique provides simultaneous collection of submicron spatial resolution infrared (IR) and Raman spectra to help more thoroughly characterize the MP particles. These complimentary vibrational spectroscopy techniques provide key insights into the identification, distribution, and amounts of contamination identified. This approach promises to bring together both IR and Raman research and researchers, to work synergistically in tackling this great analytical and environmental challenge.

05.09.04 Agglomeration of Escherichia coli with Positively Charged Nanoparticles Can Lead to Artifacts in a Standard Caenorhabditis elegans Toxicity Assay

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The increased use and incorporation of engineered nanoparticles (ENPs) in consumer products requires a robust assessment of their potential environmental implications. However, a lack of standardized methods for nanotoxicity testing has yielded results that are sometimes contradictory. Standard ecotoxicity assays may work appropriately for some ENPs with minimal modification but produce artifactual results for others. Therefore, understanding the robustness of assays for a range of ENPs is critical. In this study, we evaluated the performance of a standard Caenorhabditis elegans (C. elegans) toxicity assay containing an Escherichia coli (E. coli) food supply with silicon, polystyrene, and gold ENPs with different charged coatings and sizes. Of all the ENPs tested, only those with a positively charged coating caused growth inhibition. However, the positively charged ENPs were observed to heteroagglomerate with E. coli cells, suggesting that the ENPs impacted the ability of nematodes to feed, leading to a false positive toxic effect on C. elegans growth and reproduction. When the ENPs were tested in two alternate C. elegans assays that did not contain E. coli, we found greatly reduced toxicity of ENPs. This study illustrates a key unexpected artifact that may occur during nanotoxicity assays.

05.09.05 Effect of Polystyrene Micro/Nanoplastic Size and Surface Functionalization on Uptake and Viability of Gastric Cells

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Micro/nanoplastics (MP/NP) pollution in the environment is ubiquitous. These anthropogenic pollutants have been detected in human food and beverages, but safety information of MP/NP contaminated food is scarce. To understand the consequences of oral exposure to MP/NP, the uptake and toxicity of these particles were studied in SNU-1 human gastric cells using 50, 100, 200, 500, 1000 or 5000 nm sized and surface functionalized (animated, carboxylated) or non-functionalized polystyrene particles, as model MP/NP. Cellular uptake of 50 nm particles after 4 h treatment, was significantly higher than 1000 nm particles ($p < 0.001$), regardless of surface functionalization, as determined using flow cytometry and confocal laser scanning microscopy. Among the 50 nm particles, aminated beads localized in or around plasma membrane and were taken up significantly more (14% of total dose) than carboxylated (5.4%) or non-functionalized particles (4.7%) ($p < 0.001$). Moreover, cellular viability studies performed using alamarBlue® assay indicated that the 50 nm aminated particles were cytotoxic at a lower concentration (≥7.5 µg/mL) than other particles (≥10 µg/mL). After 4 h treatment, the intracellular pool of Caspase-8 (an indicator of early apoptosis) was significantly higher in cells treated with 5000 nm particles compared to cells treated with same surface functionalized 50 – 200 nm particles ($p < 0.05$). However, 50 nm particles induced a significantly higher proportion of cells to undergo late stage apoptosis and necrosis in 4 h compared to 1000 and 5000 nm particles, as determined by the Annexin V – propidium iodide apoptosis-necrosis assay ($p < 0.01$). As observed with cellular uptake and viability studies, the 50 nm aminated particles, caused significantly higher percentage of cells to undergo apoptosis-necrosis (29.2%) compared to 50 nm carboxylated (16.8%) or non-functionalized particles (14%) ($p < 0.01$). Overall, uptake and toxicity of polystyrene particles were highest for 50 nm, especially amine surface functionalized particles. This study demonstrated that polystyrene MP/NP can be toxic to gastric cells, which is particle size, dose and surface functionalization dependent.

05.09.06 Null Effects in Daphnia magna from Chronic Exposure to Red and Blue Polystyrene Nanoplastics

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As on plastic pollution continues to grow, it is essential to understand the effects of plastic particle exposure on freshwater species, particularly for particles reduced to the nanoscale, termed nanoplastics. A preliminary study involving 0-750 ppm polystyrene (PS) nanoplastics showed no lethal effects on *Daphnia magna* when exposed acutely. Chronic nanoplastics exposures using environmental indicator species at the base of the food web, like *Daphnia magna*, are essential for understanding the effects of plastics on long-term environmental health. In this study juvenile *Daphnia magna* were exposed to 50 nm red and blue PS Visiblex™ nanospheres at 5 ppm and 50 ppm. Each triplicate of exposures and unexposed controls contained 5 individuals that were reared for 28-days. Mortality, immobility, and reproductive output were assessed daily with feedings. All offspring were removed and placed into clean media during daily assessments and the number of offspring was recorded. After 28-days, the *D. magna* were removed from solution and imaged for growth assessment. The data collected showed no significant impact on mortality or offspring production for *D. magna* exposed to either 5 ppm or 50 ppm of 50nm red PS nanospheres. The results of the 50 nm blue PS nanospheres showed a slight increase in mortality at 50 ppm after 26-days, but this was not significant. There were no effects on offspring production among either concentration of blue 50 nm PS nanospheres. Neither the red nor the blue chronic exposure groups showed significant effects on the growth of the *D. magna*. Further investigations using a variety of different plastic types, shapes, sizes, and surface coatings should be performed to better assess the chronic toxicological effects of environmentally relevant nanoscale plastics on *D. magna*.

05.09.07 Risk Assessment of Polystyrene Microplastic in the Plankenburg River, Stellenbosch, South Africa

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Plastics are widely used in industrial, commercial, and domestic processes and activities due to their versatility. This has resulted in their occurrence in environmental compartments, including waterbodies. The concern over the ecological effects of microplastics (MPs) have been reported with evidence of ingestion and trophic chain transfer in freshwater organisms. Preliminary studies revealed the occurrence of microplastics in the Plankenburg River. Anthropogenic activities in the vicinity of the sampling points were major drivers of microplastics in the Plankenburg River. The ecological and human health risks potential of MPs in the Plankenburg River using eco-toxicological bioassays was performed. *Daphnia magna, Raphidocelis subcapitata and Tetrahymena. thermophila* were the three test models used for eco-toxicological risk assessment. Mutagenicity was assessed using Ames test in addition to probabilistic models for human health risk assessment. Dark red polystyrene plastic microspheres (10 µm) were used in the experiment as primary microplastics. Experiments were conducted to assess effects of temperature rise (0.5 °C, 1 °C and 1.5 °C) for each of the three bioassays which provided insights into the potential response(s) of the model organisms to climate change effects. Results of daphnids mortality, algal growth, and yield inhibition as well as the protozoan growth inhibition will be presented. Potential adverse effect(s) posed by microplastics to humans such as mutagenicity will be presented.

05.09.08 Developing a Species Sensitivity Distribution for Nano- and Microplastic Particles by Using Bayesian Hierarchical Modeling

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Environmental contamination with nano- and microplastic particles is an emerging global concern. The estimation of species sensitivity distributions (SSDs) is fundamental in deriving a predicted no effect concentration (PNEC) in ecological risk assessments for nano- and microplastic particles. Although properties of plastics such as the particle size can affect toxic effect concentrations, such influences have not yet been quantitatively considered in estimating SSDs for nano- and microplastic particles. Here, we developed a Bayesian hierarchical SSD based on readily available toxicity datasets by considering the influence of particle size, polymer type, and test type of medium. Results of the hierarchical SSD modeling indicated that the SSD mean was negatively associated with particle size and was lower in marine media than in freshwater media. By using the developed SSD, we will show how the hazardous concentrations for 5% of species (HC5) vary depending on the particle size and test type of media. Hierarchical SSD modeling allows us to simultaneously consider the influences of e.g., nano- and microplastic properties in estimating an SSD, thereby helping to better clarify their ecological risks.

05.09.09 Relative Risk Modeling Framework for Comparing the Contributions of Microplastics and Other Stressors Into the Adaptive Management of California Waters

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The proposed methods for estimating risk due to micro or nanoplastics have been based on the use of quotients methods, typically a comparison of the PNEC to the estimated concentrations in the environment. The PNEC has been based on specie sensitivity distributions from multiple toxicity tests with multiple test species. Other methods propose qualitative rank based estimates, based on limited toxicity and exposure data. In two instances, data from one site, the "best" characterized, is used to extrapolate to the region. It is as if history is repeating itself, tox results are generated without clear questions, plastic measurements are taken without connection to the biology, and the analysis resembles that of the 1990s and not the 21st century. The threat of microplastics is claimed to be real, but comparisons to well characterized stressors in the environment are not made. Uncertainty in the threat is poorly understood and described. We propose that the early 2020s state of the art approach to understanding risk be applied instead. Probabilistic risk assessment can be structured in order to decide research programs to address the required datasets, to collect field samples, to construct dose-response relationships and to calculate risk to endpoints that represent vital ecosystem services. Risk can be compared to better understood stressors such as pesticides, persistent organic pollutants, alterations to water quality and nonindigenous species among others. Instead of a threat by threat approach to management it is possible to integrate cause-effect pathways and make science-based decisions in an adaptive management framework. This presentation illustrates such a framework, the information requirements, the adaptive framework and benefits. This research is funded by the Metropolitan Water Districts, State Water Contractors, Delta Program and the CA Department of Pesticide Regulation and the National Science Foundation Growing Convergence Research Grant (1935018) program.

05.10 Next-Generation Toxicogenomics Tools for Chemical Prioritization and Environmental Management

05.10.01 Transcriptomics-Based Points of Departure for Fish - Use of In Silico Sub-Sampling to Inform High Throughput Assay Design

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Aquatic organisms are highly vulnerable to chemical exposure and are therefore commonly used in environmental hazard assessment. Traditional animal toxicity testing requires extensive resources and time. Alternative test methods involving high-throughput transcriptomics are being evaluated to characterize the potency and potential hazards of chemicals to aquatic organisms. However, while costs of transcriptome sequencing have decreased significantly, there is still a need to balance robustness of the assay design against the cost of data generation. The current study utilized *in silico* subsampling to help inform selection of minimum sample and gene set sizes to incorporate into subsequent transcriptomics-based assays. Individual larval fathead minnows (*Pimephales promelas*; 1 day post-hatch) were exposed to a diverse set of chemicals (three metals, three pharmaceuticals, four pesticides) for 24 hours in 1-ml, 96-deep well plates with 12 concentrations per chemical and eight biological replicates per concentration. RNA was extracted from each larval fish sample following homogenization. Sequence data and the benchmark dose (BMD) modeling software BMDExpress2 were used to calculate transcriptomic-based points-of-departure (tPODs) that can be anchored to apical responses (e.g., mortality, altered behavior). Random, *in silico* subsampling of biological replicates and the transcriptome were performed to ascertain assay parameters that generated consistent point-of-departure estimates while minimizing the use of animals and overall sequencing costs. Results suggest that assay design should include at least 10,000 transcript features and five biological replicates. Our results also identify a need to develop acceptance criteria for potency estimates based upon assessment of the overall BMD distribution and a minimum number of differentially expressed genes. These results will inform the optimal design of subsequent high throughput transcriptomics assays with fathead minnow and other ecologically relevant aquatic organisms. Reliable generation of tPODs that have reasonable, and quantifiable, levels of uncertainty based on minimum assay performance criteria is expected to aid the adoption of these approaches for regulatory hazard characterization. *This abstract neither constitutes nor necessarily reflects USEPA policy.*

05.10.02 Assessing the Toxicity of 17α-Ethinylestradiol in Rainbow Trout Using a Four-Day Transcriptomics Benchmark Dose (BMD) Embryo Assay

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Societal demands and practices result in a steady increase in the production of chemical compounds and their release into aquatic environments is causing unintended adverse effects in non-target organisms. However, current regulatory frameworks for assessing the potential toxicological hazard this ever-increasing number of chemicals poses are hampered because they are costly, time-consuming, and of significant ethical concern due to their reliance on live animal tests. Thus, there is an urgent demand for more efficient and ethical approaches in ecological risk assessment. Using 17α-ethinylestradiol (EE2) as a model compound, this study established an embryo benchmark-dose (BMD) assay for rainbow trout (RBT; *Oncorhynchus mykiss*) to derive transcriptomic points-of-departure (tPODs) as an alternative to live-animal tests. Embryos were exposed to graded concentrations of EE2 (measured: 0, 1.13, 1.57, 6.22, 16.3, 55.1, and 169 ng/L) from hatch to 4 and up to 60 days post-hatch (dph) to assess molecular and apical responses, respectively. Whole proteome analyses of alevins did not show clear estrogenic effects, while transcriptomics revealed responses that were in agreement with apical effects, including excessive accumulation of intravascular and hepatic proteinaceous fluid and significant increases in mortality at 55.1 and 169 ng/L EE2 at later time points. Transcriptomic BMD analysis estimated the median of the 20^{th} lowest geneBMD to be 0.18 ng/L; the most sensitive tPOD. Other estimates (0.78, 3.64, and 1.63 ng/L for the tenth percentile geneBMD, first peak geneBMD distribution, and median geneBMD of the most sensitive overrepresented pathway, respectively) were within the same order of magnitude as empirically derived apical PODs for EE2 in the literature. The 4-day alternative RBT embryonic assay was effective in deriving tPODs that are protective of chronic effects of EE2. This study is part of the EcoToxChip project (www.ecotoxchip.ca).

05.10.03 EcoToxXplorer: A Web-Based Platform for Comprehensive Toxicogenomics Data Analysis

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Toxicogenomics data are likely to play a key role in the transition from traditional to alternative toxicity testing methods. The costs of acquiring such data continue to drop, and an ever-increasing number of researchers and regulators would like to get involved in the world of toxicogenomics. However, these data are complex, and analyses typically require advanced programming skills and a deep knowledge of statistics and genomics resources, and as such, are usually handled by expert bioinformaticians. The objective of this project was to design EcoToxXplorer (www.ecotoxxplorer.ca) as a next-generation bioinformatics tool that is high performance, intuitive, and universally accessible to handle transcriptomics data for the purpose of chemical risk assessment and environmental management. The primary EcoToxXplorer analysis pipeline was built for analyzing qPCR data measured with custom EcoToxChip arrays from six ecological species (model organisms: Japanese quail, fathead minnow, African clawed frog; native species: double-crested cormorant, rainbow trout, northern leopard frog). The pipeline includes steps for QA/QC, filtering and normalization, differential analysis, interactive functional analysis, and report generation. The functional analysis is tox-focused, including integration with the AOPwiki and our custom EcoToxModule gene sets that were designed for high-level interpretation of toxicogenomics data. EcoToxXplorer can also process FASTQ files through a Galaxy server and microarray/RNA-seq counts tables through a NetworkAnalyst interface for the same six ecological species of regulatory relevance in North America. The current version of EcoToxXplorer is the result of continuous development since 2017 by the EcoToxChip project team. The development was organized around design-thinking principles in that we iteratively presented the tool to various user groups from academia, government, and industry for testing, and then refined it based on their feedback. This study was conducted as a part of a large-scale Genome Canada-funded project (EcoToxChip project - www.ecotoxchip.ca).

05.10.04 Transcriptomic Dose-Response Analysis in Zebrafish Embryos to Estimate Estrogenicity and Long Term Aquatic Toxicity of Bisphenol-A and Two of Its Replacement Compounds

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For many endocrine disrupting compounds (EDCs), acute exposure studies may not reveal the long-term impact of these compounds on the environment. While long-term exposure studies permit more accurate risk assessment, issues such as ethical concerns, prohibitive costs, and large sample size requirements make them unfeasible for routine testing. Recent research has shown that modeling changes in gene expression (i.e. transcriptomics) following acute exposure can be used to estimate doses that lead to chronic toxicity. Advancements in sequencing technology, in combination with high throughput in-vitro models, have made transcriptomic dose response modeling (TDRM) fast and cost effective. In the present study, we compare the transcriptomic dose response in zebrafish embryos exposed to bisphenol A (BPA), two replace 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. Embryos were exposed using a daily renewal dosing regimen from 0 hours post fertilization (hpf) to 120 hpf. Dose groups were based on a 5-point, 10-fold dilutions series for each compound, and a vehicle control (0.01% DMSO). Each replicate comprised a pool of 20 embryos, and each dose group comprised three replicates. Apical endpoints such as lethality, deformities and hatch rate were recorded for each dose group every 24h. At 120hpf, five embryos from each replicate were randomly pooled for RNA extraction and gene expression was measured using QIAseq UPX 3' sequencing. Another three embryos from each dose group were selected for a light-dark behavior assay and an Alamar Blue energy consumption assay. Dose-response analysis was conducted using fastBMD software for gene expression data, and USEPA BMDS software for apical effects. We predict that the resulting transcriptomic point of departure (PoD) for each compound would be predictive of its respective chronic PoD found in the literature and be more sensitive than the apical PoD derived from the other assays in this study. Additionally, we predict that BPAF will impact estrogenic pathways at a lower dose compared to TGSH based on their chemical structure and previous literature. 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.

05.10.05 Characterizing Toxicity Pathways of Fluoxetine to Predict Adverse Outcomes in Adult Fathead Minnows (Pimephales promelas)

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Fluoxetine (FLX) is the most common selective serotonin reuptake inhibitor pharmaceutical used for the treatment of psychological disorders such as depression and anxiety. Increased prescription use of FLX by human populations has led to its pseudo-persistence in effluent-receiving waterways. Despite emerging concerns of FLX becoming a potential threat to the aquatic environment, there is currently limited information on effects of FLX on aquatic wildlife, including its specific mechanism of action and associated toxicity pathways in fish. The main goal of this study was to identify and validate key molecular toxicity pathways that are predictive of apical endpoints induced by exposure to FLX using the fathead minnow (*Pimephales promelas*), a model species common to North American freshwater systems. Adult fathead minnows were exposed to three concentrations of FLX (2.42, 10.7, and 56.7 μg/L) and a control for 21 days. After 96 hours, a subset of fish was sampled, and liver and brain tissue were collected to characterize molecular toxicity pathways using whole transcriptome and proteome analyses. In addition, at the end of the 21-day exposure, individuals were assessed for apical outcomes of regulatory relevance including histopathology and fecundity. Differential gene expression observed in the liver of fish exposed to the highest FLX treatment and revealed dysregulation of pathways associated with biosynthesis and metabolism of fatty acids, which may be an upstream molecular response that led to lipid-type vacuolation of hepatocytes, as observed in the histology analysis. Whole proteome analysis of the same fish revealed dysregulation of PPAR signalling, which may be associated with the enrichment of lipid-related pathways observed in the transcriptome. In addition, there was an indication of pathway enrichment of transcription- and translation-related pathways in the proteome. Common dysregulated genes in the brain of the treated fish were related to cellular signalling processes that are influenced by serotonin levels and shown to be involved in reproductive behaviour and, in turn, reproductive success. This was confirmed through a significant decrease in fecundity following the 21-day exposure. Pathways to measurable adverse outcomes are complex, however, this research does provide some important clues to the mechanistic toxicity that FLX inflicts. This study is part of the EcoToxChip project (www.ecotoxchip.ca).

05.10.06 Effects of Life Stage and Route of Exposure on Transcriptomic Responses to Hexabromocyclododecane in Japanese Quail

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Early life stage (ELS) avian toxicity tests that incorporate transcriptomic endpoints are increasingly being seen as a viable alternative to traditional toxicity tests performed on adult birds. However, little is currently known about how transcriptional responses to environmental contaminants may differ between life stages and test methodologies. Here we investigate this question using hexabromocyclododecane (HBCD or HBCDD), a commonly used flame retardant that has been detected in various environmental compartments and animal species, including bird eggs and tissues. Previous studies suggest that birds are environmentally exposed to HBCD through diet and maternal transfer to the egg, and that environmentally relevant levels can negatively impact birds at both adult and embryonic life stage. In the present analysis, we describe transcriptional responses in Japanese quail (*Coturnix japonica*) liver following exposure to HBCD under 4 different scenarios: Study-1: Early life stage (ELS) exposure via a single egg injection (0, 0.73 or 10.5 µg/g egg, n = 5 per group), Study-2: adult exposure using a single oral gavage (0,100 or 1000 mg/Kg, n = 5 per group), Study-3: ELS exposure via maternal deposition after adults were exposed through their diet for 7 weeks (0, 3 or 9 µg/g of HBCD, n=6 per group), Study-4: ELS exposure via maternal deposition (exposure of mother to 0, 3 or 9 µg/g of HBCD through their diet for 7 weeks, n = 6 per group) and re-exposure of nestlings through their diet for 17 weeks (0, 3 or 9 µg/g of HBCD, n=6 per group). We used RNA sequencing to assess global *m*RNA expression in these samples and compare the different exposure scenarios based on transcriptomics data. Preliminary analyses show that for study 1 and 2, there are, respectively, 583 and 177 genes that were dysregulated, while for study 3 and 4 analyses are ongoing. This study will provide a detailed and comprehensive overview of the effects of HBCD exposure at different life stages and routes of exposure, and will increase our understanding of the potential for transcriptomics in toxicity testing.

05.10.07 Linking Altered Swimming Behaviour with Possible Dysregulation of Gene Expression in Fathead Minnow Larvae Exposed to Contaminated Groundwater

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A way to address the limitations of traditional risk assessment methods that rely on live animal testing which are time and resource intensive would be to employ mechanistic toxicology approaches. However, the general lack of linkages between effects observed at molecular and apical levels curtails the adoption of such approaches. The objective of this study was to test the effects of environmental complex mixtures on fish swimming behaviour and the correlation with effects on the expression of relevant genes. To this end, early life stages fathead minnow were exposed for seven days to increasing concentrations of groundwater collected from a legacy contaminated site. The effects on photo-dependent swimming (PDS) response of fathead minnow larvae were assessed using a behaviour box equipped with an UV visible – Infra Red camera. The results from the PDS response assessments revealed groundwater mixtures impaired the total distance moved by fathead minnow larvae under both light and dark conditions in a concentration-dependent manner. The effects on relative expression of genes will be assessed using a new tool, the EcoToxChip. The EcoToxChip assesses the differential expression of 376 genes involved in a variety of toxicity pathways. As the contaminants present in the groundwater samples were relatively unknown, we expect that using the EcoToxChip will help to determine the toxicity pathways of these mixtures. If there is a significant correlation between PDS response with results from transcriptomics analysis this study could help to increase the confidence in the use of ‘omics data for regulatory purposes.

05.10.09 EcoToxChip: A Toxicogenomics Tool for Chemical Prioritization and Environmental Management

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Here we provide an overview of our Large-Scale Applied Research Program (LSARP) grant from Genome Canada that aims to develop, test, validate, and commercialize quantitative PCR arrays (EcoToxChips) and a data evaluation tool (EcoToxXplorer.ca) for the characterization, prioritization, and management of environmental chemicals and complex mixtures of regulatory concern. In Project Phase 1, EcoToxChips have been developed for common laboratory model species representing the most important vertebrate groups in ecological risk assessment (fish-fathead minnow; bird-Japanese quail; amphibian-*Xenopus laevis*). Model species (adult and early life stage, ELS) were exposed via standardized tests to 8 environmental chemicals representative of natural resource/environment sectors of Canadian concern that cover a wide chemical and biological effect space (EE2, chlorpyrifos, benzo(a)pyrene, lead, fluoxetine, selenomethionine, trenbolone, HBCD) (Activity 1). An integrative systems approach based on functional ‘omics (combined global transcriptomic and proteomic profiling, targeted metabolomics) and physiological analyses across levels of biological organization is being applied to characterize relevant toxicity pathways that are mapped to adverse outcome pathways, AOPs (Activity 2); from this, and other resources, species-specific EcoToxChips consisting of 384 environmentally-responsive genes of regulatory concern are being informed, built, tested, and optimized (Activity 3). EcoToxChip performance is being validated (and further optimized) through our collaborators (Activity 4), and to date more than 900 EcoToxChips have been ordered. Under Activities 5-7, knowledge from Phase 1 is being translated to 3 native species (i.e., fish: rainbow trout; bird: double-crested cormorant; amphibian: Northern leopard frog). EcoToxXplorer.ca provides intuitive bioinformatics support, with a range of toxicologically-focused features (e.g., EcoToxModules; AOP gene list). To position the team advantageously with regard to the commercialization and institutionalization of the deliverables, our GE3LS research will produce and leverage social science knowledge about the phenomenon of “institutional entrepreneurship”. The anticipated socioeconomic benefits associated with the adoption of our deliverables include, more focused animal testing, improved regulatory decision-making, and cost-efficiencies. Here we provide a 60 months update of our project (www.ecotoxchip.ca).

05.10.10 Comparison of Apical Points of Departure to Transcriptomic Points of Departure in Fathead Minnow Exposures

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Each year, as the sheer amount of chemicals readily available to the market increases, so does the need for short-term and cost effective high-throughput assays that can be used to evaluate chemical potency and infer potential hazards to human health and/or ecosystems. Over the past decade, a number of mammalian studies have indicated short-term transcriptomics-based points of departure (PODs) are predictive of apical potency, often providing a POD that is within a factor of 10 of those derived from much longer-term tests. The present study generated transcriptomics-based points of departure (tPODs) for ten chemicals in fathead minnows (FHM). Larval FHM (6 days post-fertilization) were exposed to 12 concentrations per chemical, in a ½ log dilution series, for 24h in a 96 well plate format. Whole body RNA was extracted and then sequenced to evaluate whole transcriptome gene expression. Transcripts that exhibited a concentration-dependent response were subsequently fit to concentration-response models using BMDExpress2 software to determine benchmark doses. A tPOD was then calculated based on the 10^{th} centile of the distribution of benchmark doses. Transcriptomics-based PODs for three metals, three selective serotonin reuptake inhibitors, and four neonicotinoid(like) insecticides were compared against available apical effect data from the ECOTOX knowledgebase, focusing on impacts on survival, growth, and reproduction. FHM tPODs were uniformly lower than in vivo effect concentrations reported for fish, with tPODs being up to 2 orders of magnitude lower (i.e., health protective). In contrast, FHM tPODs were not always lower than apical effect concentrations reported for invertebrates, suggesting the need to include additional taxa in a high-throughput transcriptomics-based screening program. On-going research is extending these comparisons to additional chemicals and additional species to evaluate the hypothesis that high-throughput transcriptomics assays with aquatic organisms may be a viable alternative to traditional aquatic toxicity tests for ecological safety evaluations. *The contents of this abstract neither constitute, nor necessarily reflect, official USEPA policy.*

05.10.11 Linking Transcriptomic Points-Of-Departure (tPOD) to Apical Chronic Responses in Embryo-Larval Fathead Minnows Exposed to Fluoxetine

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Current methods for chemical hazard assessment face significant challenges, given the rapidly growing number of compounds of emerging concern (CEC) requiring assessment. This is because current testing strategies rely on live animal testing, which are time-consuming, expensive, and ethicaly questionable. These concerns serve as an impetus to develop new approach methodologies (NAMs) to advance chemical hazard assessment that do not rely on live animal tests. This study explored the application of molecular benchmark doses (BMDs) derived from a short-term embryo-larval fathead minnow (FHM) assay to develop transcriptomic points-of-departure (tPODs) to assess the chronic effects of fluoxetine (FLX), a highly prescribed and potent selective serotonin reuptake inhibitor ubiquitously found in surface waters. Fertilized FHM eggs were exposed to graded concentrations of FLX (measured: water, 0.19, 0.74, 3.38, 10.2, 47.5 µg/L) for 7 and 32 days. Whole body tissues were subjected to omics (transcriptomics and proteomics) and locomotor analyses (7 days), and to histological and biometric measurements (32 days). Overrepresentation analyses of both transcriptomics and proteomics data revealed significant perturbations in gene sets associated with serotonergic and axonal functions. Transcriptomics point-of-departure (tPOD) analyses estimated $omicBMD_{20} = 0.56$, $omicBMD_{10th} = 5.0$, $omicBMD_{mode} = 7.51$, and pathBMD 5.66 µg/L FLX. These tPODs are protective of apical locomotor and reduced weight effects (LOEC of 10.2 µg/L) and are well within the range of chronic apical BMDs of FLX reported in the literature. Furthermore, the distribution of geneBMDs revealed a bimodal pattern, revealing disruption of sensitive neurotoxic pathways at low concentrations and metabolic pathway perturbations at higher concentrations. This testing methodology is one of the first studies using a short term embryo assay at a life stage not considered to be a live animal under current legislations, to derive tPODs, which were protective of apical responses of FLX. This study is part of the EcoToxChip project (www.ecotoxchip.ca).

05.11 Pharmaceuticals in the Environment

05.11.01 Update on the Proposed Environmental Risk Assessment Regulatory Framework for Drugs Being Developed by Health Canada

C. Hughes, C. Whitall, Health Canada

Currently, the safety, efficacy and quality of drugs are assessed under the Canadian *Food and Drugs Act* (FDA) and the environmental risks of the drug ingredients are assessed under the *Canadian Environmental Protection Act, 1999*. The Government of Canada is introducing proposed amendments to the FDA to provide the ability for Health Canada to create a single-window notification, risk assessment, and risk management regulatory framework for the approval of drugs under the FDA that will streamline the regulatory process for industry and bring Canada's regulatory regime more in line with international jurisdictions in several key areas. This session aims to present proposed amendments to the Canadian FDA and *Food and Drug Regulations*, that will strengthen the environmental risk assessment and risk management of drugs in Canada.

05.11.03 A Framework to Prioritize Environmental Risks of Pharmaceutical and Personal Care Products from Reclaimed Water in Agro-Food Systems

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Reclaimed water irrigation introduces numerous pharmaceuticals and personal care products (PPCPs) into the agroecosystem. The ubiquitous occurrence of PPCPs in field soil, combined with the toxic, carcinogenic, or endocrine-disrupting nature of some PPCPs, raises serious concerns about their potential environmental and human health risks. However, our collective knowledge on plant uptake of PPCPs is extremely scattered and disconnected, and most studies are essentially case studies. Here we develop a tiered framework to predict PPCPs accumulation in edible tissues of food crops during reuse of wastewater. Those chemicals that have high potential to enter the agroecosystem in significant amounts are identified based on annual consumption data, human excretion rates, wastewater treatment plant removal rates and literature values. Process-based quantitative structure-property relationships (QSARs) are integrated into Trapp's models to describe the PPCPs flux from the input sources, through the soil/root interface, and into leaves/fruits, and further human exposure through dietary intakes, while a threshold is set for each process leading to the final accumulation of PPCPs in the edible tissue. A priority list of high-risk PPCPs that exhibit the greatest accumulation potential in edible plant parts is derived using the tiered framework. This framework could help predict plant accumulation of PPCPs from molecular structures and easily available physicochemical properties, and are applicable to new PPCPs or to screen high-risk PPCPs.

05.11.05 Coastal Stormwater Pond Pollutants and the Potential for Development of Antibiotic Resistance in Vibrio and Enterococcus Bacteria

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The Southeastern coastal plain is the most rapidly urbanizing region in the United States. Landscape ecology changes which increase imperviousness lead to alterations in the hydrological cycle, increasing runoff of nonpoint source (NPS) pollution. NPS runoff is discharged into stormwater retention ponds to reduce loading into coastal ecosystems. In the South Carolina coastal zone there are >21,594 retention ponds, which concentrate NPS pollution such as chemical contaminants, nutrients, and microbes, at levels much higher than in adjoining estuarine waters. Studies of legacy pollutants and contaminants of emerging concern in retention ponds indicate elevated levels of trace metals which exceed sediment quality guidelines (SQGL), in addition to the presence of emerging contaminants like clinically relevant antimicrobials. Such ponds have also been shown to contain high levels of fecal coliform bacteria. Other investigations have shown significant association between trace metals contamination in surface waters and sediments with increased rates of antibiotic resistance (ABR). Given the nature of retention ponds to isolate and concentrate pollutants like trace metals along with bacterial contaminants, these ponds may pose a public health risk from drug-resistant microbes. Even in the absence of human pathogens, affected microbes may pass on resistance genes to harmful bacteria in nearby estuaries, where they may be transferred during flood events. With the increase in extreme rainfall events that accompanies climate change, such conditions will increase in frequency and severity. Interactions of trace metals commonly found in SC ponds and estuaries (As, Cu, Zn), antimicrobial products (triclosan, ciprofloxacin, and oxytetracycline), and aquatic pathogenic bacteria (*Vibrio vulnificus* and *Enterococcus faecium*) will be discussed in terms of how they may enhance antibiotic resistance and underlying mechanisms for upregulation of resistance genes. Additionally, exposure data for *V. vulnificus* and *E. faecium* grown in the presence of antimicrobials and/or trace metals will be presented alongside resistance profiles of the organisms following single or binary exposure.

05.11.06 Time Variance of Antibiotics and Antibiotic Resistance Genes in Wastewater Effluent from a Conventional Wastewater Treatment Facility

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Antimicrobial resistance is a growing public health crisis largely driven by the presence and dissemination of antibiotics, and subsequently the antibiotic resistance genes (ARGs) which confer resistance to them. The co-occurrence of antibiotics, pathogens, ARGs, mobile genetic elements, and already resistant bacteria make conventional wastewater treatment facilities a high-risk environment for the dissemination of resistance. Due to their incomplete removal during treatment, antibiotics and ARGs are commonly discharged in treated effluent and detected in the receiving environment, such as rivers or agricultural soil and crops in the case of wastewater reuse. These sub-inhibitory concentrations are sufficient for maintaining if not expanding the prevalence of antibiotic resistance. However, there is no consensus on the abundance of antibiotics and ARGs in wastewater effluent. In this study, effluent from a conventional wastewater treatment facility in Oregon was sampled weekly for ten weeks to investigate variations in the abundance of ARGs and antibiotics in time. Presence/absence of 7 ARGs and one class 1 integron gene were determined. Relative abundance (normalized to 16S rRNA gene copies) of detectable genes were further quantified. Relative ARG abundance ranged from 4.2×10^{-1} to 2.3×10^{-5}. Concentrations of 20 target antibiotics representing major drug classes will be determined by HPLC-MS/MS. Correlations between concentrations of antibiotics and ARGs will be statistically tested to determine co-occurrence. It is hypothesized that some antibiotics will be commonly detected in all samples due to their use in the serving population and incomplete removal during treatment. ARGs are hypothesized to remain prevalent in effluent due to selective pressure from corresponding classes of antibiotics, resulting in their co-occurrence (e.g., sulfonamide antibiotics will co-occur with sulfonamide resistance). The key preliminary result from this study is that no outliers were detected from 10 sampling days regarding relative abundance of each studied ARG. This suggests that ARG discharge from a facility without combined sewer systems may be relatively consistent over time. This work will provide a better framework for interpreting variability in antibiotic and ARG data from wastewater treatment plants, as well as a broader understanding of the risk posed by antibiotics in wastewater.

05.11.07 Prioritizing Pharmaceutical and Personal Care Product Contaminants in Great Lakes Tributaries Using Risk-Based Screening Techniques

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In a 2018 study of 44 sampling sites across 16 Great Lakes tributaries, 113 pharmaceuticals and personal care products (PPCPs) were detected from a suite of 261. Sampling sites were selected to represent a gradient of land use and wastewater treatment plant (WWTP) influence. In some cases, multiple sites on the same tributary were sampled to observe changes due to source inputs. Samples were collected during low- and increased-flow periods to assess hydrologic variability. PPCPs are contaminants of emerging concern that typically lack formal water quality benchmarks. To provide a risk-based assessment of potential biological effects resulting from exposure to PPCPs, toxicity information from EPA databases (ECOTOX Knowledgebase and ToxCast) was used to develop screening-level water quality benchmarks and to compute hazard quotients (HQs) for 59 of the detected PPCPs. Ten PPCPs were designated as high priority based on HQ threshold exceedances at >10% of monitored sites: caffeine, nicotine, albuterol, sulfamethoxazole, venlafaxine, acetaminophen, carbamazepine, gemfibrozil, metoprolol, and thiabendazole. For approximately 75% (197) of the monitored compounds, the available evidence did not suggest substantial concern, according to HQs (49) or a lack of detection (148). Screening values could not be developed for 54 of the detected PPCPs due to a lack of biological effects information; therefore, these compounds represent unknown, potential threats to aquatic life. Samples collected during low-flow periods had higher PPCP concentrations than increased-flow period samples. Some of the highest PPCP concentrations and detection counts were from Lake Erie and southern Lake Michigan tributary sites. Tributary sites in northern Lake Michigan and Lake Superior had some of the lowest PPCP concentrations and detection counts. Of the watersheds with multiple monitoring sites, an upstream/downstream pair on the North Branch of the Portage River had the greatest relative difference in PPCP concentrations and detection counts. The fraction of streamflow attributable to WWTP effluent correlated positively with PPCP concentrations; however, there was substantial deviation from the relationship, indicating potential additional PPCP sources. Further research should be conducted to investigate high priority PPCPs and those for which screening values could not be developed. *The contents of this abstract neither constitute, nor necessarily reflect, official USEPA policy.*

05.11.08 Systemic Scoping Review of the Occurrence and Health Risk Antiretroviral Drugs (ARVDs) in Africa Water Resources

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Increase in the production and consumption of antiretroviral drugs (ARVD) such as HIV, COVI-19 among other developed vaccine can impact quality of the water resources due to current and previous wave's viral infections globally. The release of ARVD residues discharged into surface water; municipal drains and rivers will ultimately flows into coastal waters. Literature suggests that increase in levels of ARVDs residues in water resources will leads to both ecological and public health implication. A systematic scoping review was used to describe the extent, range and nature of published evidence between 2010 and 2020 on levels of ARVD chemicals in water resources; surface water, wastewater, drains amongst others in Africa continents. Findings reveals that majority of reports focus on therapeutic and efficiency of the ARVD, relatively fewer publications consider the levels in water resources such as potable and surface water. Additionally, limited studies were found to be conducted in low income countries relative to high income countries, South Africa recorded largest number of publications reporting various levels of ARVD in water resources relative to other countries in Africa. Other gaps identified in this research include the need for effective abatement strategy to reduce levels of ARVDs in aquatic systems. Elevated levels of ARV drugs in the aquatic environment could leads to ecological and public health implications. This research provides technical support for water and environmental protection stakeholders such as city manager, researchers, and informs policy makers in their decision.

05.11.09 Modelling the Distribution of Antibiotics in the Global River System

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Significant concentrations of antibiotics in rivers may lead to the gradual development of drug-resistant bacteria, among many other potential impacts. Still, there is a lack of observational data in the field since these substances are not typically included in routine monitoring programs, especially in developing regions. In this work, we develop a model to estimate the emission of various antibiotics and their subsequent transport in river networks at high spatial resolution and global scale, enabling first-time estimates of the surface-water concentrations of these compounds for virtually any river in the world. The transport in the river system is estimated using the contaminant fate module in the high-resolution global river routing model HydroROUT. A key component of this research is the integration of two novel datasets in the modeling approach: (1) the average levels of consumption of antibiotics for each country in the world, which are used to estimate the release of the antibiotics in each region; and (2) a global database of 58,502 wastewater treatment plants (WWTPs) - HydroWASTE; which includes enough information to geo-locate point sources and estimate the emission of the contaminant discharges into river networks. To evaluate the model, we used 217 measurements of the antibiotic Sulfamethoxazole with good results (R-squared = 0.65). The high resolution (500-meter) predictions of the model can be used in a variety of subsequent applications. First, the model can be used to identify specific areas in river networks where high concentrations of contaminants are expected and where field studies should be focused. Secondly, scientists and regulators can use the model to develop screening methods to inform the development of guidelines or regulations designed to minimize the risks associated with the environmental release of pharmaceuticals. Thirdly, governments and operators of wastewater treatment facilities can use the model to set appropriate treatment standards for individual wastewater treatment plants and to ensure that advanced treatment technologies, which are inherently resource-intensive, are deployed only in areas where they are needed. Finally, wastewater treatment technology providers can use the results to drive the development and deployment of new treatment technologies with potential global markets.

05.11.12 Heart Attack: Zygotic Exposure to Venlafaxine Disrupts the Development and Functioning of the Zebrafish Heart

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Venlafaxine, a commonly prescribed antidepressant, is a serotonin and norepinephrine reuptake inhibitor that is routinely detected in waterways receiving municipal wastewater effluents. Several studies have raised concerns that this drug may disrupt the developmental programming of non-target animals, including fish. While antidepressants are typically designed to elicit neurochemical changes leading to behavioural alterations, cardiac arrhythmia often results as a side effect in humans. We have previously demonstrated that early venlafaxine exposure disrupts the neurodevelopmental programming of zebrafish (*Danio rerio*), but whether venlafaxine can impact cardiac development in nontarget animals is unknown. We tested the hypothesis that zygotic exposure to venlafaxine will disrupt the formation and function of the developing heart in zebrafish. To this end, zebrafish embryos between the 1-4 cell stage were microinjected with either 0 (control), 1, or 10 ng of venlafaxine to mimic a maternal transfer exposure scenario. We assessed the timing of the first heartbeat, as well as basal unstimulated and stimulated (stressor and adrenergic agonists) heart rate during early development. We also investigated the long-term impact of early life exposure to this antidepressant on cardiac performance by measuring the aerobic scope in adult zebrafish. Venlafaxine causes precocious development of the heart and impacts early heart rates. For instance, at 24- and 48-hours post-fertilization (hpf), venlafaxine exposed zebrafish had elevated heart rates, but this dropped significantly below control fish at 72 and 96 hpf. The stressor-induced heart rate in larval zebrafish was also diminished by venlafaxine exposure. Also, exposure to isoproterenol, a non-selective β-adrenoceptor agonist increased the heart rate of venlafaxine treated larval zebrafish, suggesting an increase in adrenergic responsiveness. Altered cardiac performance seen in larval fish may lead to disruptions at the adult stage, as zebrafish exhibited a 40% reduction in aerobic scope. Taken together, venlafaxine exposure at early critical developmental stages disrupts heart development and performance, and this may have long-lasting effects on the swimming performance and fitness of the animal.

05.11.13 Impacts of Fluoxetine on Exploratory Behavior in Three Non-Game Fish Native to South Carolina, USA Using Fish Mazes

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Pharmaceuticals enter the aquatic environment in many ways including through wastewater treatment outflow, which can cause nontarget organisms such as fish to be exposed. Selective serotonin reuptake inhibitors are a class of pharmaceutical that is commonly prescribed and detected in surface waters. There is a lot of evidence for impacts of pharmaceuticals at point sources, and some information exists on how highly economical species such as the salmonids move materials around in watersheds from their long migrations. However, information is lacking in how non-game fish species could act as vectors to carry contaminants to new areas within a watershed. This research represents the lab portion of our study exploring how fluoxetine impacts exploratory behavior and activity using a fish maze. We collected three non-game fish species using backpack electrofishing from stream surrounding Clemson, South Carolina in the United States: Bluehead Chubs (*Nocomis leptocephalus*), Yellowfin Shiners (*Notropis lutipinnis*), and Striped Jump Rock (*Moxostoma rupiscartes*), representing different genera and functional groups. We had three treatment groups: control, low (4 ug/g), and high (50 ug/g). We injected each fish with a coconut oil/fluoxetine implant (just coconut oil for the control group) on the ventral side, anterior of the pectoral fin. On days one, three, and five following the injection, select fish from each group were placed in individual mazes to measure their activity and behavior. Fish were videotaped for 30 minutes within the mazes after which blood, liver, gallbladder, and brain samples were taken to be analyzed for the presence of fluoxetine and its metabolites. We hypothesize that the fish given the higher dosage of fluoxetine will show less activity than the control group or the fish given the lower dosage. The lab portion of this study will be used in conjunction with a mark recapture field study to hypothesize on how fish may act as vectors in contaminant movement, which could be important consideration given in trophic transfer of materials and mapping contaminant spread.

05.11.15 RNA Sequencing Reveals Potential Neurotoxicity Biomarkers in Pimephales promelas Exposed to Wastewater Effluent

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Current wastewater treatment plant (WWTP) technologies have a variable rate of removal of contaminants of emerging concern (CECs). As a result, discharged effluent results in complex mixtures of these contaminants in receiving streams that vary temporally. Among the most frequently detected CECs in WWTP effluent are pharmaceuticals. As wastewater effluent-dominated streams are becoming increasing common worldwide, this leads to pseudo-persistent exposure conditions that can negatively impact the aquatic and terrestrial organisms that live in and near these streams. Currently there is a paucity of knowledge on the neurological impacts due to exposure to complex CEC mixtures at environmentally relevant concentrations in real-world scenarios. These knowledge gaps leave incomplete assessments of the hazards and risks from exposure to such CEC mixtures as even sublethal neurotoxicity can lead to alterations in ecologically important behaviors in wildlife. To help better understand the potential real-world neurological impacts of CEC mixture exposure, we performed a 96 h *in situ* exposure of fathead minnows (*Pimephales promelas*) in a temperate-region effluent-dominated stream (i.e., Muddy Creek) near Coralville, Iowa, USA. Caged minnows were placed both 100 m upstream and 100 m downstream of a WWTP effluent outfall. After the 96-h exposure, we performed a total RNA sequencing analysis of the collected brain tissues to assess differential gene expression in male and female fish, separately. This revealed 122 unique genes for females and 111 for males that were significantly differentially expressed at the downstream site when compared against the upstream site, with only 19 such genes that overlapped amongst females and males. Functional enrichment analysis was performed on the differentially expressed genes to better understand their specific biological impacts. These brain-specific transcriptomic effects of WWTP effluent exposure in fathead minnows provides potential novel biomarkers to expand monitoring of environmental effects.

05.11.17 Characterization of Contaminants of Emerging Concern in U.S. Coastal Waters: A National Assessment by NOAA's Mussel Watch Program

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Since 1986, the National Oceanic and Atmospheric Administration's (NOAA) Mussel Watch Program (MWP) has monitored the nation's coastal waters for legacy chemical contaminants and biological indicators of water quality. In 2010, due to budgetary constraints and a shift in chemicals of national concern, the MWP began a rotational regional sampling model and began analyzing over 200 contaminants of emerging concern (CECs) such as pharmaceuticals and personal care products (PPCPs), per- and polyfluoroalkyl substances (PFAS), and multi-residue pesticides. This new regional model increases the flexibility of the program, allowing it to combine historic sites with new, targeted sites that are chosen for strategic reasons or in collaboration with local stakeholders. Since 2014, the MWP has completed regional studies in the Gulf of Maine, Gulf of Mexico, West coast and the south- and mid-Atlantic coasts. With this data, we are able to analyze the presence, distribution, concentration and mixtures of CECs that are being bioaccumulated in bivalve tissue on a nationwide scale and to compare findings across species and matrixes. Additionally, the development of land-use analyses and prioritization techniques, brings further insight into the findings and helps structure future sampling efforts. The MWP monitoring data, including the CEC dataset, provides a unique national perspective to local results and serves as baseline tool for directing more targeted management and monitoring actions at state and regional levels.

05.11.19 Fate and Risk of Methylated/Demethylated Products of Typical Pharmaceuticals in the Soil-Plant System

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Numerous studies have shown the ubiquitous occurrence of pharmaceuticals in treated wastewater (TWW) and biosolids, which are commonly used in agricultural fields for sustainable purposes. This leads to concerns over the fate and potential risks of pharmaceuticals in agroecosystems. Before and during the treatment at wastewater treatment plants, or after entry to the soil-plant continuum, pharmaceuticals undergo several common routes of transformation, including methylation and demethylation. Such transformation-induced structural modification will undoubtedly result in significant changes in their physicochemical properties as well as their biologically activity. However, there has been limited researches to evaluate the environmental consequences of these transformations. Moreover, the methylated/demethylated transformation products (TPs) of pharmaceuticals may undergo demethylation/methylation in different environments, further complicating the environmental cycling of these compounds. In this study, acetaminophen and its O-methylated product, diazepam and its N-demethylated product, and naproxen and its O-demethylated product were selected as the typical pharmaceuticals and their TPs. The possible back and forth conversions between pharmaceuticals and their methylated/demethylated products in the soil and plants were investigated. Results from this study are expected to provide information for the transformation, cycling and bioaccumulation of common pharmaceuticals and their TPs.

05.11.20 Impact of Compost and Biochar Soil Amendments on the Mobility of Emerging Organic Contaminants in Sandy Field Lysimeters

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The need to produce more food for the world's growing population had led to a greater demand for fresh water for irrigation. Unable to meet this demand, wastewater irrigation is becoming a common practice in many countries. However, the presence of emerging organic contaminants, i.e., pharmaceuticals and personal care products (PPCPs), in wastewater may represent a major risk as these contaminants may be harmful to the environment and human health. A two-year field lysimeter study (2017 and 2018) investigated the effects of soil amendments created from organic wastes (*e.g.*, table waste compost, barley straw biochar) on the fate and transport of selected PPCPs, *viz.*, caffeine, carbamazepine, DEET, diclofenac, ibuprofen, and triclosan. Biochar alone or in combination with compost was incorporated into the topsoil (0-0.10 m depth) of sandy soil. Six treatments, in triplicate, were tested in a completely randomized design (CRD): (*i*) non-amended soil (WW control), (*ii*) 1% biochar, (*iii*) 3% biochar, (*iv*) 7.5% compost, (*v*) 1% biochar and 7.5% compost, and (*vi*) 3% biochar and 7.5% compost. The impact of soil amendments on PPCP transport provided a wider understanding of the relationship between

PPCP properties (e.g., water solubility, octanol-water partition coefficient, acid dissociation constant) and their mobility in soil. The results are examined in the context of the physicochemical parameters of organic contaminants and the effects of soil amendments on soil properties such as CEC, DOC, TOC, and pH.

05.11.21 Pharmaceuticals in the Agro-Environment

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Irrigation with reclaimed wastewater exposes the agro-environment to pharmaceuticals and other organic pollutants. Once in the soil, pharmaceuticals may undergo several fate-determining processes, mainly, sorption to soil organic matter, biodegradation, and plant uptake which may introduce them into the food chain. Here we report data on 65 pharmaceuticals in irrigation water, soils, and fresh produce (leafy greens, carrot, tomato, potato, banana, avocado, and citrus) grown in commercial fields irrigated with reclaimed wastewater in Israel. All irrigation water (i.e., reclaimed wastewater) and soils were polluted with pharmaceuticals depending on the wastewater treatment and source. The number of pharmaceuticals ranged between 4 – 46 in irrigation water samples and between 1 – 21 in soil samples, at concentrations similar to other studies. Pharmaceuticals were detected in all crops (i.e., edible part >99%). In total 24 pharmaceuticals were detected in the fresh produce, ranging from low ng/g to low µg/g (dry weight). Leafy greens exhibited the highest number of pharmaceuticals at the highest concentrations reaching up to >2000 ng/g. A lower number and concentration of pharmaceuticals were detected in fruits and tuber crops. Carbamazepine and venlafaxine exhibited the highest detection frequencies in the fresh produce. Carbamazepine was detected in 95% of the leafy greens, in all citrus samples, in 99% of the analyzed avocado, in 93% of the banana samples, 79% of the analyzed tomatoes, 53% of the potatoes, and 21% of the sampled carrots. Venlafaxine was detected in leafy greens (99%), citrus (15%), avocado (37%), banana (7%), tomato (21%), and carrot (47%), but was not detected in potato samples. This study demonstrates that irrigation with reclaimed wastewater exposes the agroecosystem to traces of pharmaceuticals which may accumulate in soils and be taken up by crops. Data from this study can be used to perform a reliable risk assessment and regulations concerning reclaimed wastewater irrigation.

05.12 Soil Contaminants: Fate, Bioavailability, Environmental Toxicology in Ecological and Human Health Risk Assessment

05.12.01 Effects of Wastewater Treatment Residual Physiochemical Characteristics on Native PFAS Partitioning

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Per- and polyfluoroalkyl substances (PFAS) are a class of highly persistent contaminants that have been detected in biosolids. PFAS enter the terrestrial environment via a variety of pathways, including land-application of biosolids. The physiochemical characteristics of wastewater (WW) residuals (e.g., biosolids and sewage sludges) are known to affect PFAS release to water; thus, mobility and bioavailability may vary with different wastewater treatment plant (WWTP) processes. The release from WWTP residuals to water can be described using biosolid-water partition coefficients, thus development of predictive tools to estimate these coefficients will be useful to regulatory bodies and WWTP design. For twelve WW residuals, we measured release of partition coefficients (K_{ds}, L/kg) of a range of PFAS native to the residuals along with biosolids characteristics including total Fe and Al, oxalate extractable Fe and Al, organic matter content, dissolved organic carbon, and total protein content. WW residuals were equilibrated for 48 h at approximately 1:5 mass (g) to volume (mL) ratio in a static system at 20°C and then centrifuged to separate pore-water from the residuals. Pore-water centrifugate was then cleaned and concentrated by solid phase extraction methodology while PFAS concentrations in biosolids (both total and biosolids after pore-water equilibration) were extracted via sequential organic solvent extractions followed by soluble carbon removal by ENVI-Carb cleanup to yield mass balances. Several PFAS including perfluoroalkyl carboxylates, perfluoroalkyl sulfonates, fluorotelomer sulfonates and some sulfonamides were quantified in extracts using reverse-phase liquid chromatography quadrupole time-of-flight mass spectroscopy. Correlations between the measured physiochemical characteristics and PFAS partitioning were examined to identify key characteristics controlling PFAS release from the biosolids. Results are expected to be of high interest to academia, government, and industry and to potentially influence WWTP configuration and biosolids disposal practices.

05.12.02 Polycyclic Aromatic Hydrocarbon and Metal Contamination in Urban Playground Soils Throughout Oklahoma City

S. Hileman, Oklahoma State University / Integrative Biology

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 cPAH 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. Because schools in this metropolitan range tend to have a high degree of concern, there is the potential for oral exposure to these contaminants in sensitive age groups by way of hand-to-mouth actions involving soil. Recent data from playground soil at elementary schools throughout the Oklahoma City Area have shown elevated but varied cPAH and metal levels that may correspond with traffic sources. These sites should be further investigated to determine potential sources of contamination.

05.12.03 Public School Playground Soil Contamination: An Environmental Justice Issue?

S. Hileman, Oklahoma State University / Integrative Biology

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. This investigation examined soils from more than 130 public elementary schools across 15 school districts for levels of polycyclic aromatic hydrocarbons (PAHs) and metals. Carcinogenic PAHs 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 indicated possible issues for children attending schools with higher levels of PAHs 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.

05.12.05 Correlating Soil Nutrient Test Lead with Bioavailable Lead in Highly-Contaminated Soils Receiving Lead-Immobilizing Amendments

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Lead (Pb) is one of the most common metals exceeding human health risk guidelines for soil concentrations worldwide. Pb bioavailability is known to vary depending on soil physiochemical characteristics and, as a result, in vitro and in vivo tests exist that are used to estimate bioavailable Pb in contaminated soils. Although in vitro tests such as the relative bioavailability leaching procedure (RBALP) present simpler and more cost-effective risk assessments than in vivo methods, soil tests such as Mehlich-3, Modified Morgan, and ammonium bicarbonate-diethylenetriamine pentaacetate (AB-DTPA) extractions are extremely routine and even more cost-effective. Data are lacking examining the viability of common soil nutrient tests for assessing Pb bioavailability in soils from contaminated sites with extremely high total Pb concentrations or for sites that have received amendments, such as those containing compost, iron, and/or phosphorus, intended to immobilize Pb. Here, we examine the correlation between RBALP Pb and Pb as determined using three commonly utilized soil tests, Mehlich-3, Modified Morgan, and AB-DTPA, at one Pb-contaminated site receiving compost amendment (Seattle, WA, USA) and one extremely Pb-contaminated site receiving mixtures of compost, P, and Fe (Joplin, MO, USA). At both the Seattle and Joplin sites separately, RBALP Pb was significantly correlated with all three soil nutrient test values, regardless of soil amendment. However, RBALP was only significantly correlated with Modified Morgan and total Pb when examining the Joplin and Seattle data together, likely the result of differing controls on Pb solubility from the different sources of contamination at the two sites. These findings suggest that a diverse suite of relatively inexpensive and accessible soil nutrient test methods are likely to be correlated with bioavailable Pb at a specific site, regardless of whether Pb-immobilizing amendments have been used.

05.12.06 Shifts in Roadside Soil Microbial Communities and Responses of Terrestrial Isopods Following Overspray of Unpaved Road Chemical Treatments

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Roadside soil communities include a diverse array of microbes and invertebrates that play vital roles in nutrient cycling, soil and vegetation health, and food webs for terrestrial wildlife. These communities are also susceptible to exposure to road-related chemical stressors, such as dust suppressants and soil stabilizers. Although dust suppressants are commonly applied to unpaved roads worldwide, the effect of these chemicals on soil health and the potential toxicity to roadside organisms has been understudied. To address this gap, we exposed soil microbial communities present in a natural silt loam to two application rates of two dust suppressant products: durablend™, a magnesium chloride plus binder, and EnviroKleen®, a synthetic fluid plus binder. One application rate simulated direct overspray and the other was half the direct overspray rate. We used the same two products and rates in experimental treatment of sand substrates in 7-day toxicity tests with terrestrial isopods (*Porcellio scaber*). In natural soils, phospholipid fatty acid analysis (PLFA) indicated shifts in microbial communities with product application that persisted throughout the 28-day experiment. Treatment with durablend™ decreased the abundance of fungi, while treatment with EnviroKleen® increased the abundance of gram-positive bacteria, relative to untreated controls. Treatment with either product decreased the abundance of actinomycetes. In isopod tests, survival was reduced relative to the untreated sand at both durablend™ rates and at the direct overspray rate of EnviroKleen®. These results provide the first experimental evidence that exposure to dust suppressant chemicals can alter the composition of soil microbial communities and cause mortality of terrestrial invertebrates. The most pronounced effects occurred at the direct overspray rate, underscoring the need for responsible application procedures in the field. These results help address a general lack of information on environmental impacts of dust suppressant and soil stabilizer applications and can be used to inform environmental risk assessments of chemical treatments used in unpaved road management.

05.12.08 Physiological and Molecular Characterization of Active Fungi for Degradation of Glyphosate

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Pesticide contamination is a substantial problem in controlling agricultural pests. Understanding the physiological and molecular characteristics of naturally occurring fungi in the pesticide contaminated environment is crucial to manage glyphosate contamination. The aim of this study was to isolate and molecularly characterize soil microfungi for their physiological roles towards glyphosate degradation. Pure cultures of fungi were isolated from soil contaminated with glyphosate at Ikorodu farm, Lagos Nigeria. The cultures were grown on minimal salt agar media amended with glyphosate. The best isolates exhibiting good tolerance to the glyphosate were characterized using molecular techniques. The BLAST search indicated that the fungi belong to four *Aspergillus* species (*Aspergillus flavus* strain JN-YG-3-5, *Aspergillus niger* strain APBSDSF96, *Aspergillus fumigatus strain* FJAT-31052 and *Aspergillus flavus* strain APBSWTPF130), *Trichoderma gamsii* and *Penicillium simplicissimum*. The biodegradation study of the glyphosate by the selected fungi species showed accumulation of Aminomethylphosphonic Acid (AMPA) except for *Aspergillus fumigatus* strain FJAT-31052. This suggests that AMPA is a valid pathway for degradation of glyphosate by fungi. Annotation analysis of the partial gene sequence suggests that the strains not only possess protein coding gene clusters for glyphosate utilization but also for other physiological activities. A comparative genome analysis revealed that the genomes of the fungi were highly similar with genomes of environmental samples especially to *C. pefringes*. The GhostKOALA output confirmed that CYP2W1 gene (Cytochrome P450, fungi type) was present in *Aspergillus fumigatus* strain FJAT-31052 which was absent in genome of other fungi. The physiological and molecular characteristics of *Aspergillus fumigatus* strain FJAT-31052 clearly show that this isolate is a useful organism for managing contamination by glyphosate pesticide. Therefore genome wide assessment of this organism is recommended.

05.13 Trends in Environmental Risk Assessment of Pesticides

05.13.01 FRAGSTATS: Characterizing Spatial Interaction Between Pesticide Usage and Ecological Receptors to Inform Scenario Selection for Landscape Studies

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Landscapes are often complex and difficult to interpret, especially in relation to potential influences of pesticide usage on local ecology. Accurate spatial representation of a stressor-receptor relationship can often be challenging to characterize when using simple geographic information system (GIS) tools. FRAGSTATS is an established and robust spatial analysis program that computes a wide variety of metrics to quantify landscape structure and interaction (composition and configuration). The current study utilized FRAGSTATS to rank the relative vulnerability of the wood mouse (*Apodemus sylvaticus*) in relation to Oilseed Rape (OSR) fields for over 2000 unique 25km^2 scenarios within a single study area in Poland. The results of the analysis were used to inform a scenario selection process. We examined over 25 combinations of metrics to assess cross correlation and utility for our specific study purpose. Scenarios were ultimately characterized based on the 'Edge Density' between potential application areas (i.e., OSR fields) and wood mouse habitat derived from remote sensing (i.e., grassland, woodland). The resulting quantitative ranking of relative scenario vulnerability was suitable for scenario selection as input to the ecological effects modeling phase of the larger study. Ultimately, scenarios and model results will be submitted for regulatory review in support of pesticide product registration in Poland. This study also leveraged Python scripting to integrate FRAGSTATS within ArcGIS Pro to automate a large portion of the workflow (see separate poster for details). The analysis presented here showed that FRAGSTATS is a powerful tool that can help describe landscape structure and interaction potential. It can be efficiently implemented over large extents and multiple scenarios to support landscape-level pesticide risk assessments.

05.13.02 Towards a Facilitated Use of Modelling in Environmental Risk Assessment

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Within EU only, more than 100000 man-made chemicals are awaiting assessment of their risk to the environment. Based on statistical analyses providing toxicity indices at different tiers, risk assessment faces today new challenges to meet all expectations in terms of regulatory requirements together with the use of advanced and sound statistical methods. In particular, EFSA today recommends a clear and unambiguously identification of uncertainty sources, the use of toxicokinetic-toxicodynamic models to refine tier 2 risk assessment in particular for plant protection products acting on aquatic organisms when exposed to environmentally realistic concentration profiles and that models be documented in a transparent way ensuring reproducible results. If plenty ideas, methods and tools already exist in the academic world to meet these expectations, practitioners struggle in appropriate them for reasons mostly attributable to modellers themselves. These reasons mainly come from lacks of support: (1) to easily quantify uncertainties, then their propagation to model outputs and subsequent predictions; (2) to better accept changing paradigm using new modelling approaches often appearing as black boxes, together with a lack of support to fully perceive the concrete added-value of these novelties for their daily work; (3) to easily interpret goodness-of-fit criteria and therefore trust model results in their ability to support decisions from predictions; (4) to appropriate recent user-friendly turn-key facilities, while already recognized as automatically providing toxicity indices of interest in full compliance with regulatory guidelines and risk assessment decision criteria. Based concrete case studies dealt with a suite of convenient and relevant tools freely available within an all-in-one facility, this presentation will illustrate how the above-mentioned difficulties can be overcome to facilitate the use of models in the environmental risk assessment (ERA).

05.13.03 Developing Protective Ecological Boundaries for Pesticides

M.B. Kosnik, M.Z. Hauschild, P. Fantke, Technical University of Denmark / Quantitative Sustainability Assessment, Department of Technology, Management and Economics

Pesticide use nearly doubled from 1990 to 2018, and is projected to increase with population growth. While there are ample data on pesticides, determining their emissions and adverse effects remains challenging due to diverse application scenarios and ecosystem conditions. Protective thresholds for pesticides must be identified at relevant spatiotemporal scales with greater consideration of species' diversity and sensitivity to determine a sustainable level of pesticide pressure in the environment. We outline a three-step framework to establish regional ecological boundaries for pesticides, and discuss related challenges and ways forward. In step 1, toxicity-related pesticide pressure on ecosystems is quantified. We developed a method to prioritize pesticides (using use, persistence, mobility and toxicity) to identify those likely to cause high ecosystem damage. We used this priority list to quantify country-level impacts (applying ecotoxicity characterization factors to calculated pesticide emissions) for a set of defined pesticide application scenarios, demonstrating that the top 35 pesticides cover 60% of cumulative ecosystem damage of 255 considered pesticides, while the bottom 150 cover < 5%. In step 2, ecosystems' ability to withstand pesticide pressure (i.e. their 'carrying capacity') is defined. We integrated national data to conduct geospatial analysis of crop locations, water body characteristics, and species richness/density distributions, and compared the resulting maps at different times in a given year to highlight the spatiotemporal aspects of carrying capacity development (e.g., pesticide use and different species' vulnerability vary across ecosystems and through the year). Finally, in step 3, units and scales of the pesticide pressure (step 1) and carrying capacities (step 2) are aligned to enable comparison and define a 'safe space' wherein pesticides can be used without irreversible adverse ecological effects, and this space is allocated to entities contributing to the pollution pressure (e.g., pesticide manufacturers, farmers, etc.). We discuss methods to define and allocate the 'safe space' (e.g., to individuals based on consumption expenditures), and outline concepts to improve allocation schemes. By expanding the three-step framework, the full impact of pesticide use can be determined to protect environmental health, inform sustainable levels of pesticide use, and establish relevant protection goals from a regional to global scale.

05.13.04 Advancing Pesticide Ecological Risk Assessment Through the Use of High-Throughput Toxicity Data

S. Glaberman, George Mason University / Biology; M. Varde, George Mason University; B.R. Blackwell, U.S. Environmental Protection Agency / Office of Research and Development/Center for Computational Toxicology and Exposure

The pesticide regulatory ecological risk assessment (ERA) process has changed little over the last several decades and has traditionally relied on whole organism laboratory toxicity data. The increasing concern over animal use as well as the need to better capture lower concentration sublethal effects has led to the generation of many high-throughput assays (HTA) to estimate effects to organisms. However, given the great scrutiny that comes with shifting regulatory risk assessment, it is important to explore the value added by these new approaches. Here, we ask the following question: "what would happen in a typical agricultural use pesticide ERA in the United States if we used publicly available HTA data instead of whole animal toxicity data?" We evaluate (1) whether different types of HTAs from USEPA's ToxCast Database consistently or inconsistently over-predict, under-predict, or similarly predict traditional ERA outcomes or (2) shed new light on potential biological effects from pesticides that may be missed by a traditional ERA. The goal is to determine whether HTA data could be used to supplement, prioritize, or streamline future pesticide regulatory ERA procedures.

05.13.05 Getting BeeHave to Behave: Extending and Calibrating an Agent-Based Model of Honey Bee Dynamics for Pesticide Exposure Data Using Approximate Bayesian Computation (ABC)

D. Dawson, USEPA / Center for Computational Toxicology and Exposure; J. Minucci, S.T. Purucker, US Environmental Protection Agency / Office of Research and Development

Pesticide impacts to colonies of honeybees (*Apis mellifera*) are of increasing concern due to the importance of honeybees as crop pollinators. Honeybees have a multi-stage life history driven by a complex social system and make use of both fresh and stored food resources. This complicates predicting the influence of pesticide exposures on honeybee colonies and makes development of regulatory guidance challenging. Because of the complexity of honeybee social structure, agent-based modeling has been an attractive way to model stressors on colony dynamics. The agent-based model BeeHave was previously extended to model exposure to pesticides in pollen. In this study, we further extended BeeHave to incorporate exposure to pesticides via the nectar pathway. In addition, we used data collected from a field-based colony feeding study with the pesticide clothianidin to calibrate the model using approximate Bayesian computation (ABC). By avoiding the need for a specific likelihood function, the ABC method is well suited to calibrating complex agent-based models. Our enhanced model reasonably reproduced colony population dynamics following pesticide exposure via nectar. The ABC process also provided posterior distributions of input parameters for the model, as well as dose-response function parameters, allowing for the prediction of several key toxicological endpoints (e.g., LD_{50}, NOAEL, and LOAEL). This model will be useful for evaluating a range of potential exposure scenarios for honeybee colonies. Further, the model may serve as a valuable comparison against ongoing regulatory colony model development efforts in the US and Europe. *The views expressed here are those of the authors and do not necessarily represent the views or the policies of the U.S. Environmental Protection Agency.*

05.13.06 A Bayesian Network Approach to Probabilistic Risk Assessment of Pesticides in Rice Fields

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Today's aquatic environment is constantly exposed to various pollutants resulting from anthropogenic activities, such as the use of plant protection products in agricultural practices. The traditional environmental risk assessment is usually based on a calculated risk characterization such as a quotient representing a ratio of exposure to effects. This deterministic approach uses single values of predicted environmental concentration and predicted no effect concentration to calculate a risk quotient, and accounts for uncertainty by using an assessment factor. Contrary to this deterministic approach, we try to account for variability and uncertainty by integrating probability distributions for exposure and effects that are propagated throughout a Bayesian network model. We focus on the risk assessment of various pesticides in a representative study area and their potential risk to the aquatic ecosystem. This study is carried out for a rice field in Spain (Albufera region) and offers a transparent way of estimating the risk inside a rice paddy and drainage water. While facilitating the communication of estimates and uncertainties, the developed model can also predict the probability of several levels of the risk quotient.

05.13.07 Field Endpoints: Linking Pesticide Field Study Measurement Endpoints to Protection Goals for Pesticide Risk Assessment

J.M. Stafford, L.W. Brewer, Compliance Services International; A. Bone, Duke University / Environmental Toxicology and Risk Assessment; S. Levine, Bayer AG - Crop Science Division / Regulatory; D.E. Edwards, BASF Corporation / Ecotoxicology; A.J. Jones, FMC Corporation / Environmental Sciences; S.R. Mortensen, BASF Corporation; R. Brain, Syngenta Crop Protection, Inc.; K. Henry, NovaSource / Tessenderlo Kerley, Inc.; M. Basu, CropLife America

Field studies of pesticide exposure and effects have been conducted for decades. EPA first published guidance for such field studies in 1982, including designs incorporating aquatic organisms, birds and mammals, and aquatic and terrestrial plant species. Further information on semi-field and field testing was issued as recently as 2014, when EPA released guidance for pollinator field studies. These studies present additional opportunities for refinement and addressing uncertainties of a tiered ecological risk assessment paradigm, when needed. However, well-established laboratory-based acute and chronic effect studies that meet specific data quality requirements and which are focused on individuals remain the backbone for FIFRA ecological risk assessment and Endangered Species Act risk assessments. Field effects studies can produce data that are complex, susceptible to confounding factors, and often difficult to interpret; thus limiting their application in some risk assessment scenarios. However, carefully designed and rigorously executed field effects studies can also provide data that are superior to laboratory studies by addressing effects at the population level and above, by examining population dynamics, species diversity, species abundance, recovery, and other relevant higher-tier endpoints. Ideally, a focused field study design can maximize these benefits while minimizing the shortcomings inherent to these study designs. For example, flexibility in study design should facilitate focus on compound-specific, use-specific, target organism-specific, and/or process-specific considerations. Further, simple designs employing straight-forward endpoints to measure and interpret in field studies should be prioritized over more complex, resource-intensive designs that do not add proportional value to the risk assessment. This paper assesses the current status of field study use in pesticide risk assessment, review a number of relevant field study designs, and describe how these attributes can be aligned with assessment endpoints and specific protection goals for both FIFRA and ESA in order to better inform risk assessments.

05.13.08 Integrating FRAGSTATS, ArcGIS Pro and Excel to Automate Spatial Workflows Supporting Landscape-Level Risk Assessments

L. Insinga, C.M. Holmes, Applied Analysis Solutions, LLC; T. Schad, Bayer AG Crop Science Division / Environmental Modelling

Scenario selection during environmental risk assessments often involve large amounts of repetitious spatial data processing workflows which can be inefficient and burdensome when large portions are performed manually. Limitations of simple geographic information system (GIS) tools can further compound spatial data processing inefficiencies. FRAGSTATS is a robust spatial analysis program that computes a wide variety of metrics to quantify landscape structure and interaction (composition and configuration). The current study utilized FRAGSTATS to rank the relative vulnerability of a biological receptor in relation to agricultural fields and inform a site selection process in support of pesticide registration (see separate platform for details). The analysis exhibited highly repetitious workflows that involved several pieces of software (e.g., FRAGSTATS, Python, ArcGIS Pro, Excel) and were time-prohibitive when conducted manually. To address this, we leveraged Python scripting to integrate FRAGSTATS within ArcGIS Pro to automate large portions of the workflow. The resulting integration permitted a user-friendly execution of FRAGSTATS and subsequent post-processing, all from within the ArcGIS Pro interface that significantly reduced manual data management efforts. The analysis presented here showed that FRAGSTATS can be implemented efficiently to automate reoccurring spatially-based workflows in support of landscape-level pesticide risk assessments.

05.13.09 Weight of Evidence for Threatened and Endangered Species: A Case Study

D. Campana, Compliance Services International / Risk Assessment; L.M. Duzy, A.R. Frank, J.M. Giddings, Compliance Services International

Systematically reviewing multiple lines of information from independent sources to assess if a particular conclusion is supported through a "weight of evidence" framework is a common scientific process used to evaluate studies with conflicting outcomes. Using a weight of evidence framework has been used to evaluate the available evidence of effects of an action or activity for a variety of topics. There has been interest and effort from pesticide registrants, regulators, and the larger scientific community in recent years to refine and institute a common method under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) due to the variety of different methods used to conduct a weight of evidence. The objective of presentation is to outline and identify lines of evidence that are relevant to a weight of evidence for a pesticide endangered species evaluation under FIFRA. We use a case study to illustrate how the weight of evidence can be used to evaluate the effects of pesticides on species listed under the Endangered Species Act as part of a pesticide registration or registration review. The case study focuses on bifenthrin use and usage in cotton and assumes a risk hypothesis that the use would adversely impact the Cape Fear shiner (*Notropis mekistocholas*), an endangered fish. The weight of evidence focuses on assembling data to introduce both the proposed action (pesticide registration) and the ESA-listed species under evaluation, as well as consultation and conservation activities that together have been established on the landscape and the status of the species.

05.13.10 Critical Review and Recommendations to Improve the Quality and Reproducibility of the USEPA Chronic Mysid Shrimp Testing Guideline (CLA and CLE Joint Project)

A. Samel, FMC Corporation / Environmental Sciences; J.P. Staveley, Exponent; E. Bruns, Bayer AG, Division Bayer CropScience / Ecotoxicology; A. Olson, BASF Agriculture Solutions / Environmental Toxicology; S.K. Long, Valent U.S.A. LLC / Center for Chemical Regulation and Food Safety; D.E. Edwards, BASF Corporation / Ecotoxicology; C. Habig, Compliance Services International; T.M. Blickley, Corteva agriscience / Ecotoxicology; B. O'Neill, DuPont Crop Protection; A. Bone, Duke University / Environmental Toxicology and Risk Assessment; G. Kraetzig, ADAMA Deutschland GmbH; T.W. Valenti, Syngenta Crop Protection, Inc. / Environmental Safety; M. Habekost, BASF Corporation / Agricultural Solutions - Global Ecotoxicology; A.J. Jones, FMC Corporation / Environmental Sciences

The U.S. EPA OPPTS 850.1350 Mysid Chronic Toxicity Test (draft, 1996) is conditionally required for the registration of crop protection products in the U.S. Although this study is not required by regulatory agencies outside of North America, results have an impact on product registrations across the globe if the endpoint drives the risk assessment. The challenges with successful completion of this study design are due to a lack of detailed information on the study design and on the study conduct within the test guideline (TG). Issues with test organism performance and not meeting acceptability criteria often result in the study needing to be repeated. CropLife America (CLA) has previously identified issues concerning the language in the existing TG, including: 1) Lack of clarity about study endpoints; 2) Limited guidance for handling the G2 populations; 3) Unrealistic data quality objectives for growth and reproduction; and 4) A need to update procedures to reflect methodological improvements learned after 20+ years of laboratory experience in performing this study. Subsequently, a joint CLA-CLE (CropLife Europe) workshop was held July 14, 2020 to review issues with study design and subsequently develop recommendations for the modernization of the Test Guideline The objectives of the joint CLA-CLE project team are to identify: 1) areas of inconsistency between the OPPTS and ASTM TGs; 2) areas in the OPPTS test guideline that require additional clarification (e.g., G2 mysids); and 3) areas in the OPPTS TG that are not practical or have uncertain scientific relevance with respect to the objectives of the study. This presentation will provide an overview of the project's progress and we look forward to discussions with all interested parties.

05.13.11 Compilation and Statistical Analysis of Pollen and Nectar Pesticide Residue Levels: Applications to Tier 1 and Refined RUDs for Pesticide Risk Assessment

L.W. Brewer, Compliance Services International; T.B. Fredricks, Bayer CropScience / Global Regulatory Sciences; S. Hinarejos, Sumitomo Chemical Agro Europe / AgroSolutions Division International; M.A. Feken, Syngenta / Ecological Risk Assessment; T.A. Joseph, Landis International, Inc. / Regulatory; V. Sa, Dow AgroSciences; W. Warren-Hicks, ECOSTAT

Honey bee dietary risk assessment of pesticides requires knowledge of the residue levels in nectar and pollen, either following foliar application to crops, trunk/stem injection, soil application or seed treatment. Current Tier 1 bee risk assessment in the United States relies on an exposure estimation and risk assessment model called BeeREX. This model uses a Residue Unit Dose (RUD) approach to estimate residues in nectar and pollen based on the upper□bound pesticide residue values from US Environmental Protection Agency's (USEPA) T□REX model (version 1.5) of residues measured on a variety of plant matrices assembled for the purpose of dietary risk assessment in birds and mammals. Specifically, the RUD for 'long grass' residues are used within BeeREX as a surrogate for residues in nectar and pollen. In comparison, European Union (EU) Tier 1 risk assessment uses a database of nectar and pollen residue data. The USEPA has recently received residue study data from several applicants that can be used to adequately describe the distribution of pesticide residues that occur in pollen and nectar relative to application rate, method of application, and crop. By combining the EU and USEPA variety of plant matrices, especially nectar and pollen databases a statistically refined estimation of RUD values can be calculated. The calculated nectar and pollen RUD values will then inform the BeeREX model with exposure data relevant to the bee risk assessment.

05.13.13 Aquatic Risk Assessment of Binary and Ternary Mixtures of Organophosphate Insecticides in Washington State Surface Waters

J. Ryan, Montana State University / Natural Resources Assessment Section

Ecological risk assessments often do not consider potential additive, synergistic, or antagonistic effects from mixtures of chemicals and instead typically base risk on a single chemical. In the last decade, more tools and models have been developed to consider the interactive effects of chemicals within a mixture when conducting risk assessments. Therefore, this study uses actual environmental concentrations measured in 2018 and 2019 from the Washington State Department of Agriculture's Surface Water Monitoring Program. Aquatic risk from exposure was assessed from chlorpyrifos, diazinon, and malathion (as individual chemicals and as binary and ternary mixtures) using the concentration addition model. These pesticides were selected because they have a common mechanism of toxicity, are frequently detected in surface waters in Washington, and were recently evaluated in a biological opinion by the National Marine Fisheries Service. All detected concentrations of chlorpyrifos and malathion, assessed as individual chemicals, exceeded the predicted no effect concentration, indicating potential for adverse effects on aquatic life. Further, risk quotients for all binary and ternary mixtures were greater than one, also indicative of potential for adverse effects on aquatic life. In all samples containing a mixture, the maximum cumulative ratio suggested that a single insecticide contributed >50% of the overall toxicity of each mixture. Based on the individual and mixture risk quotients, chlorpyrifos and malathion were the primary drivers of the toxicity of each mixture.

05.13.14 Agrochemical-Laden Fugitive Particulate Matter from Beef Cattle Feedyard Causes Mortality Among Apis mellifera and Osmia lignaria (Honeybees and Mason Bees)

F.B. Green, Texas Tech University / Environmental Science and Studies; E.M. Peterson, P.N. Smith, Texas Tech University / Environmental Toxicology

The agriculturally intensive Southern High Plains (SHP) region of the United States is a mosaic of concentrated beef cattle production, row crops, and some native grasslands. Beef cattle feeding operations use a variety of pesticides (e.g., neonicotinoids, pyrethroids, macrocyclic lactones) to reduce pests and increase yields. These chemicals become suspended in air via large amounts of particulate matter (PM) that is dispersed into the surrounding environment by wind. Recent characterization of this fugitive PM has revealed that substantial amounts of pesticides are thus leaving feedyard boundaries and distribute into adjacent environs. For example, PM emanating from a single feedyard can contain up to 28,900 ng permethrin per cubic meter of air. As pesticide-laden PM settles out of the air it can become deposited onto planted crops, flowers and other native flora, and pollinators that are embedded in nearby landscapes. Many of the chemicals commonly found in fugitive feedyard PM are highly toxic to pollinators such as honeybees or mason bees. Given the documented global decline of pollinators and potential risk to pollinators occurring near beef cattle feedyards, we sought to evaluate environmentally relevant exposure of pollinators to agrochemical-laden PM under field conditions. We conducted *in-situ* exposures of honeybees and mason bees near feed yard boundaries in which bees were placed in cages both downwind and upwind of each of three feedyards as well as in negative control cages, repeated four times during months when pollinators are active. Treatments placed downwind of feedyards produced bees coated with PM and resulted in significantly higher mortality as compared to those placed upwind of feedyards and controls. These data inform pollinator risk assessments in the SHP by estimating pollinator mortality due to a previously uncharacterized source of pesticide exposure.

05.13.15 Pesticide Presence, Land Use, and Honey Bee Hive Health in North-Central Oklahoma

C.C. Klase, J.B. Belden, K. Baum, Oklahoma State University / Integrative Biology

Honey bees (*Apis mellifera*) are pollinators of a variety of crop species. With declines in the number of commercial hives, understanding the factors that impact honey bee survival and health is crucial to maintaining agricultural production. Managed honey bee hives are frequently placed on or near crop fields to provide pollination services, potentially exposing them to pesticides. Exposure to pesticides can occur through two primary routes: external/physical contact (which can be assessed through surface residue of pesticides) and diet (which can be assessed through pollen collected by honey bees). Our objective is to determine whether the presence of pesticides influences honey bee health across three land use types (canola crop, winter wheat crop, and grassland). We are evaluating if pesticides occur in pollen from hive pollen traps and on the surfaces of surrounding wildflowers and crops and, if so, at what concentrations. We are also assessing whether hive health (measured as weight changes and *Varroa* mite parasite loads) is affected by pesticide presence and concentrations. We placed three hives at 24 study sites (12 sites in 2019, four near each land use type, and repeated at different site locations in 2020) in north-central Oklahoma. Hives remained at the sites from March to early October, and samples were collected three times during that time period (late April/early May during peak canola bloom, July during post-canola harvest, and late September). During each sampling period, we collected samples to assess pesticide surface residue of crops and wildflowers, pesticides in pollen from hive pollen traps, hive weights, and *Varroa* mite loads in each hive. We expect that as quantities and concentrations of pesticides increase, the *Varroa* mite loads will increase and the honey bee hive weights will decrease. We also expect that honey bee hives at grassland sites will consistently have greater weight increases and fewer parasites compared to honey bee hives at winter wheat sites and canola sites, although hives at canola sites will initially gain the most weight due to floral resource availability during peak canola bloom.

05.14 Understanding Exposure to Per- and Polyfluoroalkyl Substances: Building a Foundation for Future Action

05.14.01 Bioaccumulation of Per- and Polyfluoroalkyl Substances (PFAS) in Resident Fish in the Columbia Slough, Oregon

E.B. Nilsen, U.S. Geological Survey / ORWSC; J.A. Field, Oregon State University / Environmental and Molecular Toxicology; D. Muensterman, Oregon State University / Chemistry; S. Payne, I.R. Waite, US Geological Survey / OR WSC

A major source of toxic per- and polyfluoroalkyl substances (PFAS) is aqueous film forming foams (AFFF) used in firefighting and training at airports and military installations, but they have many additional sources, being widespread in consumer products. We collected fish in July 2019 and July 2020 from a reach of the Columbia Slough in Oregon that is adjacent to, and receives storm runoff from, Portland International Airport and the Portland Air National Guard base, which house historic fire training pits. We also collected fish from an upstream reference reach of the slough. We dissected fish in the field and collected muscle, blood and liver samples. Samples (0.3-0.5 g mass) were spiked with 33 stable-isotope labeled PFAS standards and extracted with acetonitrile and EnviCarb cleanup following National Institute of Standards and Technology (NIST) standard reference material (SRM) 1946 methodology developed using Lake Superior fish tissue. Extracts were analyzed for 50 target PFAS by liquid chromatography - quadrupole time-of-flight. The final analyses will include screening for additional suspect PFAS. Extraction and solvent blanks were all below the limit of detection. Analyses are underway, and we will present data from multiple fish species, reaches, and collection dates. Data analyzed to date indicate bioaccumulation of PFOS, with PFOS concentrations ranging from 3,700 - 43,000 nanograms per gram (ng/g); 2,800 - 19,000 ng/g; and 45 - 900 ng/g in fish blood, liver and tissue, respectively. These concentrations are, in most cases, substantially higher than the 2.2 ng/g PFOS determined for the NIST standard reference material certificate of analysis. In addition, FHxSA ranged from 230 - 36,000 ng/g; 150-6,000 ng/g; and 32 - 410 ng/g, in fish blood, liver, and tissue, respectively. Also, Cl-PFOS has been detected quite frequently. Finally, all carboxylic acids detected correspond to linear PFCAs, which likely indicate fluorotelomer degradation whereas sulfonates and sulfonamides signals were observed to be branched and linear species, likely indicating an electrofluorination origin. Understanding PFAS bioaccumulation in fish and food webs in key to assessing impacts of these substances on human and ecological health.

05.14.02 Rapid Screening Method for Per- and Polyfluoroalkyl Substances in Consumer Products

H. Whitehead, University of Notre Dame / Chemistry and Biochemistry; G.N. Peaslee, University of Notre Dame / Department of Physics

Analytical measurements of per- and polyfluoroalkyl substances (PFAS) are complicated by the large number of PFAS found in both commercial and industrial products, as well as the wide variety of applications in which they are used. Contributing to this issue is the time, expertise, and cost of performing both traditional extractions and subsequent targeted analysis techniques that restricts the number and kind of samples that can be measured. A rapid screening method using particle-induced gamma-ray emission (PIGE) spectroscopy has been used to complement the traditional analytical techniques for PFAS analysis such as targeted liquid chromatography tandem mass spectrometry (LC-MS/MS) and gas chromatography mass spectrometry (GC-MS). Consumer products are first screened for elevated total fluorine using PIGE and those with elevated levels are selected to undergo chemical extraction and targeted analysis. The combination of PIGE as a rapid screening tool with confirmatory analysis generates an analytical process that allows for the measurement of samples with a wide variety of matrices and PFAS present. Utilization of this analytical process for the measurement of PFAS in a variety of sample types including cosmetics, textiles and fish tissue will be discussed. Ongoing efforts to measure the occurrence of PFAS in consumer products where fluorinated polymer process aids (FPPAs) are used in their manufacture will be described as well. These products include artificial turf grass as well as plastic bags and packaging films that show elevated levels of total fluorine. The measurement of PFAS due to the use of FPPAs and the extent that these PFAS might contribute to further human and environmental exposure are currently unknown. The development of this analytical process allows for better estimation of the amount and kinds of PFAS present in products including identifying products where the knowledge of PFAS use is limited. These estimations are critical for researchers and regulatory bodies alike when determining the potential environmental and human exposure from continued use of PFAS in consumer goods and products.

05.14.04 Short and Medium Chained Chlorinated Paraffin's in Lake Trout Form the Great Lakes

B. Alipour Parvizian, Clarkson University / CAARES

Chlorinated paraffins (CP) are complex mixture of synthetic chlorinated n-alkanes with diverse physicochemical properties depending on chlorination degree. These mixtures have been widely used since 1930s as flame retardants and exceeding annual global production of 1 million tons in 2009. Depending on the alkane chain, CPs have been grouped into short (SCCP), medium (MCCP), and long (LCCP) chain. SCCPs have mainly used in metalworking applications, leather processing, rubbers, textiles, sealants, paints, and coatings as FR. MCCP and LCCP are used as plasticizers in flexible polyvinylchloride (PVC) and also as industrial metalworking fluid. Since 2012 a limited ban has been set on the SCCP in EU and both SCCP and MCCP concentration in the environment have been monitored since 2013. The ban was followed in 2017 by adding SCCP (>48%) to annex A for elimination for certain applications. As part of the Great Lakes Fish Monitoring and Surveillance Program SCCPs, MCCPs and LCCPs were determined in lake trout and the spatial and temporal resolution will be discussed to assess the impact of these chemicals on the Great Lake Region.

05.14.05 In-Depth Characterization of PFAS in Wastewater, a More Comprehensive Analysis

T. Mcknight, Eurofins TestAmerica; A. Patterson, Eurofins Environment Testing America; C. Neslund, Eurofins Lancaster Laboratories Environmental, LLC

Current analytical methodologies for measuring PFAS in the environment have led to a series of compromises in terms of sensitivity, selectivity, and capturing the whole PFAS picture. As the investigation and treatment of sites contaminated with PFAS matures, there is a growing interest in determining the contributions of different sources to the overall contamination and understanding the true mass of PFAS present. Conventional methods can measure a discrete list of compounds on the order of 70+ PFAS analytes with sensitivity in the part per trillion range. Many additional PFAS are not determined as discrete compounds due to the lack of analytical reference standards. There are methods which aim to capture additional non-discrete PFAS mass such as the Total Oxidizable Precursor (TOP) Assay and Adsorbable Organofluorine (AOF) by combustion ion chromatography (CIC), each with their own set of compromises. These methodologies have previously been compared and evaluated for strengths and weaknesses. This research aims to take the comparison and subsequent evaluation of PFAS analytical tools one step further with the inclusion of Non-Target Analysis (NTA). Utilizing a Liquid Chromatography, Quadrupole Time-of-Flight mass spectrometer (LC-QTOF-MS), we can expand upon the results from the previously studied methods to identify what the PFAS “dark matter” is comprised of and what we might be missing with all three of these commercial approaches. Management of PFAS impacted wastewater has far reaching implications for our environment. Identifying the extent of contamination, the sources of PFAS discharges, and the transformation of PFAS through this process have not been well characterized in the U.S. In order to tackle this challenge, we will require many tools in our analytical toolbelts to make informed decisions about source control, treatment, and exposure risks. To that end, influent and effluent wastewater samples will be analyzed for a targeted list of 75 PFAS compounds using LCMSMS and GCMSMS analysis. The same samples will be processed using the TOP Assay procedure in addition to AOF-CIC. Lastly, the samples will be analyzed in ESI-positive and ESI-negative mode via the Non-Target Analysis. With these data we will demonstrate how the various techniques can complement each other or where they fall short of certain objectives.

05.14.06 Factors Affecting the Migration of Perfluorooctanesulfonic Acid (PFOS) from Waste Into Groundwater in Engineered Containment Facilities

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Diffusion and leakage through holes in liners are the two primary contaminant transport mechanisms for the escape of contaminants from municipal solid waste landfills to the environment for landfills lined with modern composite liners. These landfills typically have a composite liner comprised of a high density polyethylene (HDPE) geomembrane liner over a geosynthetic clay liner (GCL) but little is known about the diffusion characteristics of these liners. Past research has indicated that HDPE geomembrane liners are more resistant to diffusion of organic compounds than linear low-density polyethylene (LLDPE) liners. The paper discusses a range of plastic materials including HDPE and LLDPE currently being examined with respect to the diffusion of per- and polyfluoroalkyl substances (PFAS) through geomembranes. To provide an upper bound estimate of the diffusion characteristics for landfills, this paper examines the diffusion and permeation coefficients of perfluorooctanesulfonic acid (PFOS) through linear low density polyethylene (LLDPE) geomembranes. The method of testing and the range of PFAS being tested is discussed but attention is then focused on the migration of PFOS through LLDPE based on almost three years of testing. This paper examines the likely transport of PFOS through a typical liner system and makes some comparisons with typical regulatory limits for impact on groundwater assuming initially that the only transport mechanism is diffusion. The paper then discusses the other potential transport mechanism for PFOS and that is leakage through holes in the geomembrane, and examines the effect of various hole configurations on the potential impact of PFOS on groundwater.

05.14.07 Analytical Considerations Regarding Per- and Poly-Fluorinated Alkyl Substances (PFAS) in Test Solutions for Toxicological Exposures

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Driven by their ubiquity in the environment, aquatic toxicity testing of PFASs is rapidly expanding to determine their potential ecological impact. Environmental sampling studies of certain PFASs suggest their unique physical-chemical properties complicate analytical quantification. This in turn raises concerns about how the design of aquatic toxicity tests with PFASs may alter exposure concentrations. Another potential complication is ensuring that the analytical method used to measure these chemicals is accurate and precise. To address these concerns, behaviors of PFASs under laboratory conditions used in conventional toxicity exposures are examined. Specific issues evaluated include: purity of commercially-available PFASs, sorption of PFASs to food and exposure chambers, and stratification of PFASs within the water column. To address variable effects from chain length and functional group, perfluorinated alkyl sulfonates and carboxylic acids with carbon chain lengths from 4 to 10 were used.Findings suggest that PFAS exposure concentrations consistently agree with nominal values across a variety of toxicity test conditions. However, work thus far has elucidated some potential issues. LCMS analyses of two independent commercial sources of perfluorohexane sulfonate (PFHxS) showed approximately 1% contamination with perfluorooctane sulfonate (PFOS). This PFOS contamination may complicate toxicity testing with organisms that are far more sensitive to PFOS than PFHxS, such as *Chironomus dilutus*. Another concern is that initial investigation into the effects of foods used in toxicity testing has suggested measurable effects on PFAS concentrations. For example, the combination of foods used in *Hyalella azteca* toxicity tests showed concentration dependent reductions in aqueous PFOS as high as 20%. Overall, results show consistent PFAS exposures in laboratory toxicity tests is achievable, although findings are presented with implications to ensure test design and analysis of exposure concentrations accommodate the unique obstacles that arise from certain PFASs.DISCLAIMER: The views expressed in this poster are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

05.14.08 Analysis of the Relative Influence of Variables Affecting Per and Polyfluoroalkyl Substances (PFAS Bioconcentration or Bioaccumulation Factors [BCFs/BAFs]) in Fish

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Fish bioconcentration factors (BCF) and bioaccumulation factors (BAF) for per- and polyfluoroalkyl substances (PFAS) that are reported in the empirical data are highly variable. The wide variability in PFAS BCF/BAF values presents challenges for determining which values are most appropriate to use for environmental risk assessments for fish, as well as for human health risk assessments with contaminated fish consumption exposure pathways, especially when site specific data are not available. Numerous variables are thought to influence PFAS BCF/BAF values, including (but not limited to) fish species, trophic level, diet, life stage, size, PFAS concentration in environmental media (water, sediment, food), type of water body (freshwater versus marine, lentic versus lotic), temperature, pH, and study methodology. However, no comprehensive review of these variables, as well as their relative effects on BAC/BAF values, currently exists. In the present study, we conducted a literature review of the available fish BCF/BAF values for perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), and perfluorohexanesulfonic acid (PFHxS), compiled data on the variables potentially influencing BCF/BAF values from each study, and conducted a principal components analysis to determine which variables are most influential to PFAS uptake in fish. Understanding the influential factors driving PFAS uptake in fish is critical to generating more accurate estimates of the potential ecological and human health impacts of PFAS contamination.

05.14.11 Ambient Water Quality Criteria for PFAS: Variation in State Approaches and Assumptions

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In the absence of national ambient water quality criteria (AWQC) for PFAS, several states have developed their own AWQCs for human health and ecological receptors, and several other states are in the development process. Perfluoroalkyl sulfonate (PFOS) and perfluoroalkyl carboxylic acid (PFOA) are the principal focus, however, other PFAS are under consideration. The AWQCs derived for protection of human health (HHAWQC), which consider ingestion of fish and water, are lower than the AWQCs derived for protection of aquatic life and wildlife receptors. There is considerable variation in the criteria, reflecting a lack of consensus on toxicity, as well as different assumptions about exposure and environmental behavior, and differences in policy. For PFOS, the HHAWQCs derived by states vary over 200-fold from a low of 0.05 ng/L (Minnesota, Proposed) to 12 ng/L (Michigan, Final). The HHAWQC calculation uses several parameters, four of which represent the greatest variability in input values used by states in criteria development: toxicity factor, fish consumption rate, relative source contribution (RSC), and bioaccumulation factor. Toxicity factors vary over 10-fold, depending on critical endpoint and point of departure selected and uncertainty factors applied. Fish consumption rates vary approximately 5-fold depending on the study and target population (e.g., recreational versus subsistence angler). The RSC varies 4-fold, reflecting differences in the amount of PFAS exposure that is assumed to come from non-water sources. There is also significant variability in the bioaccumulation factors used, reflecting differences in the studies and species selected, as well as the statistic used (mean versus upper percentile). With the exception of Florida, which used a probabilistic approach incorporating distributions for key exposure parameters, states have used a deterministic approach to calculate their criteria. The presentation will provide insight on key differences in the approaches and assumptions that have been used by states to derive their AWQCs and discuss some of the main areas of uncertainty, as well as the lack of sensitivity of current analytical methods to achieve the lowest criteria. The technical basis for key assumptions will be discussed and areas of potential over-conservatism identified based on current toxicity and exposure data.

05.14.12 Novel Perfluoroalkyl Ether Acids in Homegrown Blueberries and Blackberries from a PFAS-Impacted Community in North Carolina

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In the Cape Fear River basin of North Carolina, novel perfluoroalkyl ether acids (PFEAs) such as hexafluoropropylene oxide-dimer acid (commonly known as GenX) are receiving attention because they are widely detected in private wells and public water systems. Apart from drinking water, diet is another important exposure route. In PFAS-impacted communities, homegrown fruits and vegetables may take up PFAS from contaminated irrigation water and/or soil, but the uptake of PFEAs and other per- and polyfluoroalkyl substances (PFASs) into local produce remains unclear. Extraction methods for the determination of PFAS in food have been developed for traditionally studied perfluoroalkyl acids (PFAAs), but methods for novel PFEAs are lacking. In this study, an extraction and clean-up method was developed for the quantification of 46 PFASs, including 14 PFEAs, in twelve blueberry and blackberry samples. The extraction workflow includes sample homogenization and extraction using 0.01 M ammonium hydroxide in methanol. Mass-labeled internal standards were added, as available, prior to extraction, which included vortexing, ultra-sonication and centrifugation in triplicate, followed by solid-phase extraction (SPE) using WAX cartridges to reduce matrix interferences. In method validation, satisfactory performance was achieved with recoveries of 35 PFASs (including 10 and 9 PFEAs in blueberry and blackberry samples, respectively) within 70-130%. Recoveries of 9 additional PFAS were within 40-180%. Twelve blueberry and blackberry samples from five field sites (collected between 2015 and 2019) were extracted and analyzed using liquid chromatography−tandem mass spectrometry (LC−MS/MS). Elevated PFEAs were detected with concentrations ranging from 0.05 to 12.4 ng/g wet weight. Perfluoro-2-methoxypropanoic acid (PMPA), perfluorodioxahexanoic acid (PFO2HxA) and perfluoromethoxyacetic acid (PFMOAA) were the dominant PFEAs with maximum concentrations of 12.4 ng/g, 3.9 ng/g and 2.3 ng/g, respectively. These results suggest that produce grown in PFAS-impacted communities can be an important exposure route.

05.14.13 Do Mussels Bioaccumulate the Forever Chemical, PFAS, Differently Than Other Aquatic Organisms?

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In this study we investigated the bioaccumulation of PFAS compounds in the freshwater Pond mussel, *Ligumia subrostrata*. We focused on the freshwater mussel selected because there are very few studies reporting the uptake of PFAS in freshwater mussels and mussels are an important of food webs where PFAS occur. PFAS have been found to bind to proteins and colloids in surface water systems and, therefore, accumulation of PFAS is likely to differ based on organism feeding and behavior. In this study we determined the bioaccumulation kinetics of freshwater mussels in a controlled laboratory study. The laboratory study has focused on determining bioaccumulation kinetic parameters following exposure to PFHxS, PFOS, and PFDA at 10 ug/L and PFUdA at 1 ug/L during a 14-day uptake exposure and a 7-day elimination period. Bioconcentration factors (BCF), calculated as the ratio of PFAS in mussel tissue to water concentration, at 7 days were determined for PFXxS (0.24 ± 0.08), PFOS (7.73 ± 1.23), PFDA (4.80 ± 1.21), and PFUdA (84.0 ± 14.4). Uptake and elimination rate constants as well as time to steady state were determined to provide input to food web models. In this study we found bioaccumulation of these four PFAS chemicals in Pond mussel is similar to uptake reported for other aquatic species.

05.14.14 In Silico Prediction of Fate and Risk-Determining Properties of Per- and Polyfluoroalkyl Substances (PFAS)

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Per- and polyfluoroalkyl substances (PFAS) are the class of persistent bioaccumulative toxic chemicals (PBTs) that currently is of greatest concern among all emerging contaminants. The risks of PFAS to human and ecological health arise from their global distribution as non-point source contaminants, but also from point-source contamination from their presence in aqueous film-forming foams (AFFFs) used in firefighting at military facilities, airports, etc. To provide a complete and accurate environmental risk assessment of the continuously growing number of diverse PFAS families, it is necessary to develop predictive models for PFAS physico-chemical properties (PChPs). This study presents a suite of four molecular-structure-based in silico methods for the prediction of fate-determining PChPs, including octanol-water partition coefficients (K_{OW}). The major challenge in predicting PFAS PChPs is the handling of their ionized form as these charged-species are believed to be predominant under most environmental conditions, especially for PFAS with acidic moieties given their low (estimated) dissociation constants (p*K*a ~ 0.5). The models presented here have been evaluated based on their ability to reliably estimate the solvation of a calibration set of diverse anionic molecules in water and other various solvents and, subsequently, on their performance to predict a validation set of PFAS PChP measured data. Preliminary results show promising results for all four methods with prediction errors of approximately 0.6 and 0.8 log units for the calibration and validation sets, respectively. The different modeling approaches also reveal insights into the major mechanistic factors (e.g., p*K*a and carbon chain length) determining the behavior of PFAS between water and other phases such as octanol, commonly used as a surrogate for organism lipid, and soil organic carbon. These modeling tools may eventually provide reliable PChP estimations for large libraries of PFAS helping to predict their mobility across environmental phases more efficiently than using experimental approaches.

05.14.15 Evaluating Per- and Polyfluoroalkyl Substances (PFAS) in Alligators and Assessing the Corresponding Lipid Alterations

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Per- and polyfluoroalkyl substances (PFAS) are manmade chemicals utilized in household and industrial applications due to their chemically inert, thermally stable, and hydrophobic properties. These characteristics however are detrimental and cause PFAS to be extremely stable, leading to bioaccumulation, biomagnification and bioconcentration. Therefore, with each environmental release or spill, public health concerns rise about the safety of their drinking water and the species living in the water. Alligators are apex predators, which offer an important resource for PFAS exposure analyses as they are subject to both bioaccumulation and biomagnification. Over their approximately 30-year life span, alligators can provide insight into how long-term exposure that humans may experience affects health. Additionally, since alligators have robust immune systems, the immunotoxicant effects of PFAS can be further analyzed. To study PFAS levels in alligators, we analyzed serum from 15 different alligators from Florida and 45 different alligators from North Carolina using untargeted liquid chromatography (LC), ion mobility spectrometry and mass spectrometry measurements. PFAS identifications were first performed in a targeted manner with Skyline by matching the LC-IMS-MS features to a library containing over 100 PFAS standards. Machine learning and Kendrick mass defects were then utilized for the untargeted identification of potential PFAS. Since PFAS exposure has also been associated with lipidomic changes, lipid analyses were also performed on the alligator serum. The PFAS and lipid associations were then compared to provide a better understanding of if lipid alterations were occurring in alligators with differing amounts of PFAS in their serum.

05.14.16 Evaluation of Extraction Techniques for Per- and Polyfluoroalkyl Substances and Application to a Grocery Store Food Survey and Exposure Assessment

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Per- and polyfluoroalkyl substances (PFASs) - impacted water and soil have been identified as a key uptake pathway for plants. They raise more concerns now not only because of widespread applications of PFASs but also considering that plants are located at the basic trophic level and will lead to further PFASs bioaccumulation in food webs. We have identified, and implemented common PFAS extraction methods for plants, dairy, and tissues, comparing these methods on PFAS identification and quantification qualify. The optimized extraction methods were used to conduct a grocery store food survey including fresh and canned vegetables, muscle and liver tissue, fresh and powdered milk, and canned baby food. The survey also included a comparison of organic vs. inorganic and washed vs. unwashed produce. A recently published model was used to generate daily dietary exposure intake (mg/kg_{bw}-day) based on PFAS concentrations detected in food. The model included Monte Carlo simulations to generate daily vegetable consumption (g/kg_{bw}-day) and body weight (kg) by age group. Daily exposures were compared to human health toxicity reference dose values (RfDs) published by the United States Environmental Protection Agency (USEPA), the Agency for Toxic Substances and Disease Registry (ATSDR), the New Jersey Drinking Water Quality Institute, and Minnesota Department of Health. PFBA (1.17-2.22 ng/g dried), PFOA (0.15-1.52 ng/g dried) and PFHxS (4.55 ng/g dried) were detected in the foods surveyed. The maximum detection was PFOS in turkey liver, and PFOA was the most frequently detected. Comparison of washed vs. unwashed concentrations demonstrated that washing can reduce PFAS concentrations, in some cases to levels below detection, which can reduce human exposure. These results also elucidate whether PFAS originated from uptake (not removed by washing) vs. surficial application (e.g., pesticides). Daily PFHxS Exposure Intake (EI) for children 1-2 years of age dropped from 1.93 mg/kg_{bw}-day, which exceeded the ATSDR RfD of 0.019 mg/kg_{bw}-day, to non-detect by washing radish roots. Thus, washing is a basic step that can mitigate PFAS exposure risks associated with fresh produce. Other PFAS EI values exceeded RfDs despite washing including PFOA (inorganic lettuce) in the 1-2 and >20 year-old groups. As these results represent only a limited number of foods from a single store, additional work is needed to understand and mitigate dietary exposure risks.

05.14.17 Measurement of N-Alcohol/Water Partition Coefficients for Nonionic Perfluorocarbons

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Per- and poly-fluorinated compounds (PFAS) are a group of chemicals created by humans, that have become a growing concern in the environment. To predict and model the movement of PFAS chemicals through the environment, partition coefficients such as n-octanol-water (K_{OW}), organic carbon-water (K_{OC}), and Henry's law constants (H) are needed. Measurements for K_{OW} are limited for PFASs and using the OECD-123 Slow Stir method, we have measured the log K_{OW}s of perfluoronaphthalene (3.52 sd=0.01, n=2) and perfluorobiphenyl (4.59 sd=0.023, n=4). Measurements are underway on three aliphatic fluorocarbons perfluorodecalin, perfluoroctane, and perfluorodecane. The measured K_{OW}s will be compared to predictions from a variety of predictive methods including EPISuite, OPERA, ACD, and Chemaxon. n-Butanol/water partition coefficients (K_{BW}) are also being measured to determine if the K_{OW}-K_{BW} pairs reside on the linear free energy relationship between these partition coefficients developed using non-fluorinated organic chemicals. This abstract does not necessarily reflect USEPA policy.

05.14.18 Method Optimization for the Extraction of Per- and Polyfluorinated Substances from Biosolids

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Per- and polyfluorinated substances (PFAS) are anthropogenic compounds that are resistant to degradation and are oil and water repellent. This has led to the use of PFAS in applications like surfactants, nonstick cookware, firefighting foam, and food packaging. Due to environmental and human health concerns, there has been increased scientific and regulatory scrutiny of PFAS in various environmental media. PFAS are not efficiently removed from wastewater treatment plants, and concentrations of some PFAS increase through the treatment process due to the conversion of some PFAS, known as precursors, into different, terminal PFAS degradation products. As a result, release and/or land application of effluent and biosolids can reintroduce PFAS into the environment. There is a need to understand the composition and concentration of PFAS in residuals such as biosolids, but the complex nature of the matrix leads to challenges in extraction and analysis that are not completely addressed in published analytical methods. The objectives of the current study were to i) extract representative biosolids from multiple treatment facilities in the United States using published methods and ii) optimize the extraction solvent, extract cleanup, and sample preparation approaches that yield the most favorable internal standard and matrix spike recoveries. Biosolids were extracted using previously published basic methanol (1% ammonium hydroxide), acetonitrile, and sequential basic and acidic methanol protocols. Following extraction, the project evaluated dispersed envi-carb, envi-carb cartridges, and liquid-liquid extraction cleanup techniques. The impact of a lipid precipitation and removal step was also evaluated. Lastly, samples were prepared in water-methanol, and in water-methanol-isopropanol mixtures in order to optimize preparation approaches. Recoveries of 23 mass-labelled PFAS, and 40 unlabeled PFAS standards were monitored in order to evaluate internal standard (IS) and matrix spike recoveries, respectively. Recoveries of PFAS were influenced by multiple factors including biosolid type (e.g., sludge, pelletized or cake form) and source, analyte, and cleanup method. Overall, the extraction & cleanup methods initially yielded similar IS recoveries but employing a lipid removal step led to the greatest improvements in IS recovery. On average, the efficiency of IS recoveries doubled with removal of lipids from the extracts indicating that matrix has a great influence on the analysis of biosolids. The matrix spike recoveries for majority of the analytes were within range 50-150%. Data analysis is ongoing, but to date, extraction with basic methanol and employing dispersed envi-carb cleanup, lipid removal, and analysis of water-methanol mixtures yields the most favorable IS and matrix spike recoveries.

05.14.19 Bioaccumulation and Trophic Transfer of PFAS in a Simulated Terrestrial Food Chain

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Per- and polyfluorinated substances (PFAS) enter the soil via pathways such as biosolids-application, use of fire-fighting foams, chemical and industrial activities, and consumer goods usage. PFASs are highly persistent in the environment and the potential exists for terrestrial biota to accumulate PFAS, which may result in exposure of higher trophic level organisms, including humans, to these compounds through consumption. However, trophic transfer of proteinophilic compounds such as PFAS has not been extensively studied and the degree to which plant-accumulated PFAS will be transferred to herbivorous consumers is unclear. Here, we exposed *Solanum lycopersicum* (tomato) plants to a suite of 7 different PFAS, including 4 carboxylic acids (PFOA, PFHxA, PFHpA and PFDA) and 3 sulfonates (PFBS, PFHxS and PFOS). Exposed leaf tissue was then fed to *Manduca sexta* (tobacco hornworm) caterpillars for up to 1 week, after which caterpillars were fed clean tomato tissue for up to 1 week. While patterns of uptake and elimination were similar between the different PFAS, PFOS bioaccumulated in the hornworms to a higher concentration, featuring approximately 5-fold higher assimilation efficiency (AE) than other PFAS tested. Bioaccumulation and trophic transfer factors (TTFs) were positively correlated with PFAS carbon chain length for both sulfonates and carboxylic acids. This result suggests that although recently published work has demonstrated that shorter chain PFAS are more readily accumulated in plants, shorter-chain PFAS may also be more readily eliminated by higher trophic level consumers.

05.14.22 Application of Presaturation Equilibrium Dialysis Binding for Various PFAS to Plasma, Tissue, and Breast Milk

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Per- and polyfluoroalkyl substances (PFAS) are a large group of synthetic chemicals used in commercial household products and industrial applications. Due to their stability and prevalent use, PFAS have come under scrutiny as toxic compounds in the environment and for human health. The growing number of PFAS has precipitated the need to apply high throughput, predicative assays that can be informative for PFAS pharmacokinetics. Free drug concentration is a critical parameter for the pharmacokinetic (PK)/pharmacodynamic (PD) relationship of drugs/chemicals in the body and plays an important role in distribution and elimination. This is particularly important for several PFAS with low intrinsic clearance that are highly bound to plasma and tissues. Herein, we applied presaturation equilibrium dialysis binding, a new high throughput assay used in the drug development process to compare relative binding affinities for PFAS. Recent studies have shown the trends and affinity of PFAS to different matrixes such as diluted albumin and diluted plasma/serum. However, the current literature has not explored the undiluted physiologically relevant fraction unbound (fu) values of whole plasma/tissue, which are used to correct for free drug concentration. We evaluated the fu values of 16 PFAS against human, rat, and mouse plasma, homogenates for rat liver tissue, and human breast milk. The results showed a wide fu range of 9.6E-6 to 2.1E-1 in plasmas and 5.1E-6 to 3.1E-1 in rat liver. When comparing the molecular weight (MW) and fu for all PFAS, the R^2 values for the exponential trendlines showed relative values between was 0.90, 0.94, 0.98, 0.94, and 0.79 for human plasma, rat plasma, mouse plasma, rat liver and breast milk, respectively. When we further partitioned our data via head groups and look at only perfluoroalkyl carboxylates, the trendline R^2 value between MW and fu shifted to 0.97, 0.99, 0.98, 0.95, and 0.81 for human plasma, rat plasma, mouse plasma, rat liver, and breast milk respectively. The PFAS within the study have shown to increase their binding capacity with an increase in the number of carbons in their fluorinated carbon chains against all tested matrixes. To our knowledge, this is the first study to explore fu of highly bound PFAS against whole plasma/tissue utilizing the optimized Presat Binding Method.

05.14.23 PFAS in Air: Sampling and Identification of Persistent Contaminants

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Per and polyfluoroalkyl substances (PFAS) are a larger class of fluorinated organic chemicals of environmental concern. PFAS compounds are heat and stain resistant and have been in used globally since the 1940s when discovery of their unique properties made them ideal for a wide range of applications. Items we use everyday including carpets, clothing, fabrics for furniture, paper packaging for food, non-stick cookware and other consumer products all contain PFAS. PFOA and PFOS were the most extensively produced and subsequently the most studied of these chemicals. The rapidly evolving regulatory landscape, especially in drinking water and wastewater, has been driving the research into this topic. Regulators and scientists worldwide are now also turning their attention to how PFAS is transported and introduced into air, and it is linked to the exposure pathway for humans. Current analytical techniques to tackle this application are not optimised, with lack of standardized methodologies and require further investigation. The analysis of these compounds in air has become increasingly important, longer chain PFAS have begun to be phased out in many parts of the world. These compounds are being replaced by newer, more volatile, shorter chain (< C_8) PFAS compounds, which have limited information on environmental fate, health effects and due to their volatility are more likely to be in higher concentrations in air. This presentation will highlight the use of innovative sampling and analytical processes based on pre-concentration using thermal desorption coupled with GC-MS. One source of PFAS in air is disposal of chemicals and products containing PFAS which are incinerated. This process ensures the compounds do not end up in landfill or water courses but if the incineration process is incomplete PFAS will be released into the air. Once in the air PFAS compounds can travel large distances and contaminant ground water and soil.

05.14.24 A Comprehensive Testing Protocol for Over 100 Native and Isotopically Labelled PFAS Compounds in Drinking and Surface Water by Triple Quadrupole LC/MS

L. New, M. Giardina, T. Anumol, F. Mavandadi, C. Gan, E. Parry, Agilent Technologies, Inc.

Per and polyfluoroalkyl substances (PFAS) are chemicals widely used in consumer products and industry due to their unique and desirable chemical properties. Due to widespread usage and environmental persistence, legacy PFAS are ubiquitous in the environment and new fluorochemicals are being found in the environment frequently. USEPA, ASTM and ISO standard methods continue to evolve to meet this change, expanding target lists to incorporate emerging compounds and lowering detection limits as more is learned about the impact of these compounds. These changes put laboratories under pressure to develop expanded methods quickly to stay relevant. Here, a comprehensive testing protocol using solid phase extraction and LC/MS/MS was developed for the analysis of more than 100 native and isotopically labeled PFAS in water, with the intention of accelerating and simplifying routine laboratory testing is described. A solid phase extraction (SPE) approach was optimized for the extraction of these compounds in aqueous matrices such as drinking water and surface water. Compound separation was achieved on 1290 Infinity II LC System fitted with a PFC-Free HPLC Conversion Kit and analyzed with Agilent 6470 QQQ. An in-house verification study was performed to demonstrate that the developed method was appropriate for the analysis of PFAS in the selected water matrices with regard to sensitivity, precision, and recovery. Method detection limits were determined using ultrapure water samples and ranged from 0.14 to 14 ng/L for 60 PFAS. The interbatch precision and recovery for these 60 analytes in drinking water were within the acceptable limits of 2.2 to 16.7% RSD and 76 to 119%, respectively. The interbatch precision for 60 PFAS in surface water ranged from 1.6 to 19.9% RSD with recovery of 72 to 120%. This confirmed the method applicability for a routine and more comprehensive analysis to allow an expanded scope of PFAS testing in these two water matrices.

05.14.25 Development and Demonstration of Volatile and Semi-Volatile Per- and Polyfluoroalkyl Substances (PFAS) GC-MS Methods on Select NIST Reference Materials

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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 other biota. As a result, routine monitoring methods, method development, and commercial analysis 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, 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, are developed for analysis by three individual acquisition types and on two different instruments. Acquisition types include: electrospray ionization gas-chromatography mass spectrometry (EI GC-MS), positive chemical ionization GC-MS, and GC-tandem mass spectrometry (GC-MS/MS). The objective of this work is to demonstrate the efficacy of the methodology and to add value to existing SRMs. Three SRMs from NIST, SRM 2585 Organic Contaminants in House Dust, SRM 2781 Domestic Sludge, and SRM 1957 Organic Contaminants in Non-Fortified Human Serum, were selected for quantitative evaluation of GC amenable PFAS as a demonstration of the newly developed methods. Each of these materials represents a different analytical compartment, and all three materials have reported concentrations of ionic PFAS by LC-MS/MS on their Certificates of Analysis. The observed volatile PFAS concentration in the selected SRM materials are subsequently evaluated across instrumentation and across acquisition modes to evaluate concentration accuracy and precision. These volatile PFAS measurements add value to the volatile PFAS knowledge-base and provide needed methodological, analytical validation materials.

05.14.26 PFAS in Archived Samples and Contemporary Marine Biota From Rhode Island, USA

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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 possible spatial and temporal patterns related to collection. Further, we used these data to gauge the potential for human exposure due to the consumption of commercially and recreationally important species. Contemporary sampling efforts also allowed us to determine current concentrations in species collected within Narragansett Bay, including the subsequent potential for ecological and human risk.

05.14.27 Evaluation of the Dietary Toxicity of Per and Polyfluoroalkyl Substances in Zebrafish

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Per and Polyfluoroalkyl Substances (PFAS) are ubiquitous in the environment, detected in environmental samples and consumer products. Due to their numerous chemical structures and unique chemical properties, it is challenging to predict the toxicity of specific PFASs. Humans are commonly exposed to PFASs by consuming contaminated food and water. Perfluorohexanesulfonamide (FHxSA) and 6:2 fluorotelomer sulfonate (6:2 FTS) were selected to determine the effects of dietary PFAS exposures in zebrafish. These PFASs were selected because both were developmentally toxic in an early life stage screen and are commonly detected in environmental samples. Tropical 5D Zebrafish (165 fish per treatment) were fed a contaminated diet of 1 ng/g or 100 ng/g from 17 days post fertilization to 60 days old. At the end of exposure, the control and 100ng/g PFAS exposed fish were subsampled, to measure the PFAS concentrations in the whole fish and organs (heart, liver, brain, muscle) using LC/MS. At 90 days of age, 32 females and 32 males per treatment were subjected to behavior assessments. Social behavior was evaluated by grouping two females and two males together followed by video analysis to calculate cohesion. Fish from each group were also evaluated for hyperactivity, response to startle, and for their ability to learn. Preliminary results indicate that neither PFAS produced significant differences in any of the measured behaviors suggesting that long-term dietary exposures to FHxSA or 6:2 FTS are of low risk. Future studies will include unbiased histological analyses to determine if either PFAS is associated with cellular anomalies.

05.14.29 Application of Standard Fate and Transport Modeling Approaches for Evaluating PFAS Compounds in Groundwater

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Per- and polyfluoroalkyl substances (PFAS) used in numerous consumer products and industrial applications make their way into the environment through multiple sources. Vastness of the chemical group due to thousands of distinct PFAS and their widespread uses have led to their ubiquitous occurrence in the environment. These emerging contaminants are receiving increased attention due to their persistence and toxicity to environmental and human health. Several small and large-scale monitoring programs have shown widespread presence of these compounds in air, surface and groundwater, and soil media. USEPA has been prioritizing ongoing work to better understand and eventually reduce the potential exposure and risks caused by these chemicals. 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 environmental exposure 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. HYDRUS and PEARL models were used to simulate PFAS measured under controlled conditions. As a case study, these models were then applied to simulate a PFAS chemical in a study area in the northeastern U.S. Results from modeling were compared with available groundwater monitoring data for the study area. Practical utility of the standard modeling approaches for application to address the PFAS challenges over small and large geographies will be discussed.

05.14.30 Per- and Polyfluoroalkyl Substance Uptake Into Earthworms (Eisenia fetida) Exposed to Mixtures of Biosolids Compost and Reference Soil from Guam

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Per- and polyfluoroalkyl substances (PFAS) were detected in biosolids from wastewater treatment facilities and biosolids composted and mixed with native (reference) soil from Guam. An assessment on low-level concentrations of PFAS in Class A biosolids compost was conducted to evaluate potential risks from this compost when used for ornamental landscaping and erosion control applications. PFAS concentrations in biosolids compost were compared to residential screening levels from the United States and Australia to initially assess the potential risk to humans who consume home-grown produce growing in soils where biosolids compost has been applied. PFAS concentrations in compost exceeded the residential soil screening level with home-grown fruit/vegetable consumption. To further evaluate residential application of the Guam biosolids compost, a study of PFAS bioaccumulation in earthworm (*Eisenia fetida*) was performed. The earthworm tissue results were used to model a potential dietary exposure scenario (chicken eggs) for residents of Guam. Following the ASTM E1676-12A protocol, a 28-day earthworm bioassay was performed with five treatments (three replicates each) of soil with varying mixtures of biosolids compost. Treatments included: (1) 100 percent compost, (2) formulated control soil (ASTM protocol formulation), (3) 20 percent compost/80 percent reference soil, (4) 35 percent compost/65 percent reference soil and (5) 50 percent compost/50 percent reference soil. All soil treatments and composites of depurated (24 hours) earthworm tissue were analyzed for PFAS using isotope dilution and LC-MS/MS with modified EPA Method 537. Perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) were analyzed for both linear and branched isomers. Measured concentrations of PFOS and PFOA were used within a human health risk model that estimated consumption by domestic chickens, maternal transfer of PFOS and PFOA to eggs, followed by human consumption of chicken eggs. Earthworm tissue concentrations were also used in ecological risk models to assess potential dietary intake by resident species. Risk models with PFOS and PFOA uptake determined from Guam compost and soils indicated that a mix of 35 percent compost with 65 percent native soils are unlikely to impact resident wildlife.

05.14.31 Stability and Reactivity of Per- and Polyfluoroalkyl Substances in Solvents Relevant to Environmental and Toxicological Analysis

C. Zhang, A. McElroy, North Carolina State University / Civil, Construction and Environmental Engineering; H. Liberatore, U.S. Environmental Protection Agency / Chemistry; N.M. Alexander, D. Knappe, North Carolina State University / Civil, Construction and Environmental Engineering

Per- and polyfluoroalkyl substances (PFASs) are widely used anthropogenic chemicals. For environmental and toxicological analysis, it is important to understand the stability of PFASs, including novel per- and polyfluoroalkyl ether acids (PFEAs), in commonly used solvents. In this study, we investigated the effects of PFAS characteristics, solvent type, water-to-organic solvent ratio, and temperature on the stability of 21 PFASs including 18 PFEAs. None of the studied PFASs showed measurable degradation in deionized water, methanol, or isopropyl alcohol over 30 days; however, some PFEAs degraded in the polar aprotic solvents acetonitrile, acetone, and dimethyl sulfoxide (DMSO). PFEA degradation followed first-order kinetics, and first-order rate constants increased with increasing temperature and with decreasing water-to-organic solvent ratio. Monoethers containing a carboxylic acid functional group adjacent to a tertiary carbon degraded more rapidly than multiethers, in which the carboxylic acid moiety was adjacent to a -CF2- group. Using high resolution mass spectrometry, we determined that hexafluoropropylene oxide-dimer acid (HFPO-DA or GenX), HFPO-trimer acid, and HFPO-tetramer acid were stoichiometrically converted to Fluoroethers E-1, E-2, and E-3, respectively, in aprotic solvents. PFEA degradation results highlight the importance of solvent choice when preparing dosing solutions and performing extractions for environmental and toxicological assessments of PFEAs.

05.14.32 Total Organic Fluorine Analysis for Nontargeted Per- and Polyfluoroalkyl Substances (PFAS) Exposure Monitoring: Impacts of Method Variability on Data Interpretation

H.L. Lord, Bureau Veritas Laboratories / Environmental R&D; L. Khachatryan, F. Mo, Bureau Veritas / Inorganic Analysis

PFAS are introduced to and exist in the environment as complex mixtures. Often the nature and quantities of PFAS in a product are unknown and once released, compositions are further altered. Environmental processes result in both the production of degradation products from larger PFAS through microbial oxidations and an altering of mixture composition during migration due to variable partitioning to soil and water. This complexity highlights the need to understand potential PFAS mixture effects at the site of exposure in ecological risk assessments. Ankley G.T. et al. (2021) have recently commented with regard to ecological exposure characterization that: "Monitoring efforts should consider not just parent chemicals, but precursors, degradates, and metabolites. Accomplishing this effectively would require use of both targeted and nontargeted analytical techniques for monitoring." While targeted PFAS analytical techniques are relatively mature the development of practical nontargeted techniques is more recent. Various approaches to Total Organic Fluorine (TOF) analysis have been proposed as solutions for such nontargeted analysis, and among these Combustion Ion Chromatography (CIC) has seen considerable interest. Recent efforts for improving the sensitivity of CIC for environmental samples has resulted in different versions of the technique, employing a range of sample pre-treatment strategies and fractionations. Most notably, the Adsorbable (AOF) versus Extractable (EOF) organic fluorine approaches have emerged as leading contenders. The former uses a carbon-based sorbent for pre-treatment whereas the latter uses a sorbent more commonly employed for sample pre-treatment prior to PFAS LC-MS/MS analysis. Further variations on the EOF method involve how the sorbent is rinsed prior to extract elution and how the extract is further processed prior to CIC analysis. TOF analyses from the same sample processed by these different approaches to pre-treatment may produce different results. If TOF results are to be used in support of ecological risk assessment, a better understanding of the impact of these different processing approaches is warranted. This presentation will describe the results of a study we have conducted to evaluate the types of data variability observed between AOF-CIC and EOF-CIC with different extract processing approaches. Conclusions about potential impacts on PFAS exposure monitoring will be proposed.

05.14.33 Framework for Assessment of Uptake of PFAS in Fish at Contaminated Sites with a Case Example from Australia

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PFAS are highly persistent in the environment and often detected in humans and wildlife. Concerns regarding potential ecotoxicological and human health risks associated with PFAS in fish have emerged, with many laboratory and field studies conducted to understand PFAS bioaccumulation in fish. However, reported bioaccumulation factors (BAFs) and bioconcentration factors (BCFs) are highly variable across different PFAS. Moreover, for each PFAS, BCFs and BAFs are influenced by several factors, including but not limited to fish species, trophic level, diet, life stage, PFAS concentrations, and type of water. We conducted a comprehensive literature review of empirically derived factors of uptake of PFAS in fish and assessed available modeling approaches to estimate uptake. Based on this review, we developed a framework to evaluate and select BAFs and BCFs for PFAS in fish for site-specific risk assessment. We also present a detailed case study of the application of the framework for the determination of transfer factors for perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), and perfluorohexanesulfonic acid (PFHxS) for four freshwater fish species at a site in Western Australia. This framework can be used by stakeholders to assess potential ecological risks and human health risks associated with PFAS exposure from consumption of fish and, where needed, develop site-based screening levels and remedial goals.

05.14.34 Determination of Legacy and Emerging PFAS in Drinking and Surface Water in West and Central Florida Environments

M.B. Fatowe, X. Li, N. Soares Quinete, D. Cui, Florida International University / Chemistry and Biochemistry

Poly- and perfluoroalkyl substances (PFAS) are a group of anthropogenic compounds made of a hydrophilic head group and a hydrophobic carbon backbone which is specially structured to replace part or all the hydrogens with fluorines. PFAS are extensively used in surfactants, water repellent sprays, adhesive and coatings, firefighting foams and many other products due to their high thermal and chemical stabilities. These compounds are extremely persistent pollutants and are widely observed in the aquatic system, flora, and fauna worldwide. Its ubiquitous presence in the surface and drinking water supply and significant adverse health effects observed in animals, associated with its bioaccumulation potential, pose big concerns. In April 2021, millions of gallons of wastewater were dumped into Tampa Bay by Piney Point phosphate mining facility which highlights the contamination that Florida water sources face from toxic and noxious chemicals, which can contain high PFAS levels. This catastrophe directly impacts West and Central Florida due to the significant volume of tourists that visit these areas and utilize Florida's beaches, rivers, canals, and naturally, drinking water. The current study focuses on the detection of legacy and emerging short-chain PFAS substitutes, at low parts-per-trillion (ppt) levels by liquid chromatography–mass spectrometry (LC-MS/MS) to assess PFAS in West-Coast and Central Florida environments. A solid phase extraction (SPE) procedure followed by LC-MS/MS has been applied for the determination of PFAS in tap water collected from different counties (Hillsborough, Lee, Sarasota, and Collier) and in surface water from popular tourist sites such as Tampa Bay, Fort Myers, Naples and adjacent west-coast rivers and canals to the Gulf of Mexico. Such knowledge on PFAS contamination in Florida is needed to provide information about the occurrence, composition and distribution of PFAS in water sources which are critical to human health. In addition, the knowledge gained on emerging organic pollutants detected in our drinking water will facilitate the development of guidelines and procedures on monitoring PFAS contamination in Florida and provide detailed information for local and regional executive agencies related to land use and water quality.

05.14.35 Comprehensive Assessment of PFAS on Occurrence, Composition, and Seasonal Variation in Drinking and Surface Water in South Florida Environments

X. Li, N. Soares Quinete, D. Cui, Florida International University / Chemistry and Biochemistry

Polyfluoroalkyl and perfluoroalkyl substances (PFAS) are a group of synthetic chemicals extensively used in household and industrial products for over 60 years. Due to their widespread use and superior stability, PFAS are found ubiquitous in surface and drinking water worldwide. It poses big threat to aquatic life and human health given their bioaccumulation feature and significant adverse health effects observed in animals and human. Although the most prevalent PFAS, PFOS and PFOA, have been phased out in the U.S., the increasing emission from the alternative short chain fluorinated compounds is still a big concern, such as four carbon-based perfluoroalkyl sulfonates and carboxylic acids (PFBA, PFBS), CF3 or a C2F5 based perfluorinated ethers ("GenX" and "ADONA"). The impact of those alternative compounds is largely underestimated due to lack of studies. In Florida, PFAS have been found in surface water, sediment, and aquatic animals. However, the systematic information on PFAS occurrence and distribution in South Florida, which includes the populous Miami metropolitan area, the Florida Keys, and the pristine Everglades, is still quite unclear. The current study, therefore, focused on 1) the development of an accurate and sensitive method able to detect 30 PFAS, including legacy PFAS and emerging short-chain PFAS substitutes, at low parts-per-trillion (ppt) levels by liquid chromatography–mass spectrometry (LC-MS/MS), 2) A two-year comprehensive assessment of 30 PFAS on the occurrence, composition, spatial distribution, and seasonal variation in drinking water from metropolitan counties (Miami-Dade, Broward, and Palm Beach) and surface water from different water bodies in South Florida, such as the Everglades, the Keys, and the Biscayne bay and adjacent canals, such as Miami River, Little River, Biscayne C8.The results on current PFAS contamination status in South Florida will provide the information needed for local and regional executive agencies related to water quality, further facilitating the development of guidelines and procedures for pollution control and reduction in South Florida.

05.14.36 Biotransformation of 8:2 Monosubsituted Polyfluoroalkyl Phosphate in Human Fecal In Vitro Suspensions

S. Peskett, A. Rand, Carleton University / Chemistry

Polyfluroalkyl phosphate esters (PAPs) can be found throughout modern society due to their numerous commercial applications. This is concerning, given the ability of PAPs to be metabolized into fluorotelomer alcohols (FTOHs) and other fluorinated organic compounds of environmental and human health concern. The metabolism of PAPs has been shown to occur in mammalian liver and intestine, however metabolism by the gut microbiome has not yet been investigated. In this study, human fecal samples were used to represent the gut microbiome to test for the metabolism of 8:2 monoPAP. *In vitro* testing was completed by incubating the fecal samples at 37□ with 8:2 monoPAP (concentrations ranging from 400-7000nM) up to 60 minutes. The reactions were then terminated and the samples prepared for GC- and LC-MS analysis. Metabolites of interest were the immediate hydrolysis product of 8:2 monoPAP, the 8:2 fluorotelomer alcohol (8:2 FTOH), and 12 additional metabolites shown to form from 8:2 FTOH in both oxic and anoxic conditions. The Michaelis-Menten kinetics of 8:2 monoPAP transformation by gut microbiota were compared to those of human S9 liver and intestine fractions both of which have active levels of hydrolyzing and oxidative enzymes that transform 8:2 monoPAP. Based on preliminary data it is hypothesized that the gut microbiome plays a notable role in the metabolism of PAPs, given the significant transformation of the 8:2 monoPAP to 8:2 FTOH. The additive impact of the gut microbiome on host detoxification and/or activation of xenobiotics, is important and this study can help to inform risk assessment for continued and future use of PAPs in everyday household products.

05.14.37 The Fluoropolymer Polytetrafluorethylene (PTFE) Is Durable by Design and Thus Neither Readily nor Inherently Biodegradable

T. Ruwona, W.L. Gore & Associates, Inc.; B. Henry, W.L. Gore & Associates, Inc. / Toxicology; N. Timmer, Charles River Laboratories / Environmental Sciences

Since several organic fluorinated compounds have been detected in the environment and biota, global regulators have begun debating the risk management of per- and polyfluoroalkyl substances (PFAS). Durability is a desirable property for many products in commercial applications such as medical devices, aerospace, and pharmaceutical manufacturing. The long-term endurance of the product in these applications is critical to their intended function and high societal value. As risk is a function of hazard and exposure, we argue that persistence (i.e. durability) of a substance alone cannot, by definition, present a risk to health or the environment in the absence of hazard. Polytetrafluoroethylene (PTFE), a high molecular weight (MW) fluoropolymer, is durable by design, and does not degrade, break down or leach toxic transformation products under standard processing conditions or in the environment. As a result, we propose that PTFE does not pose a hazard to biota or the environment. To experimentally assess potential hazards of PTFE made without PFOA, we initiated a battery of environmental fate studies per OECD and EPA Guidelines. The testing paradigm is based on our knowledge of PTFE (manufacturing processes; chemical, physical, and thermal properties) and existing standard test guidelines for environmental fate, to address potential hazard concerns of PTFE and its potential leachables. We have previously shown that PTFE is stable under environmentally relevant temperatures. We hereby present data confirming that PTFE is neither readily biodegradable as per OECD Guideline 301B “Ready Biodegradability: CO_2 Evolution Test (Modified Sturm Test)” nor inherent biodegradable as per OECD Guideline 302C “Inherent Biodegradability: Modified MITI Test (II)”. Both test designs were modified with proper negative controls and extensive replicates to increase the statistical power. We therefore conclude that PTFE does not appear to break down into lower MW products that might pose environmental and ecological risks.

05.14.38 Targeted Aanalysis and Suspect Screening of Several Pesticides for PFAS

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Targeted analysis for 24 PFAS was conducted on 10 pesticide formulations used on a United States Department of Agriculture crop research field. PFOS was found in 6 of the 10 formulations with concentrations ranging from 3.92 – 19.2 mg/kg. Further analysis of soil and plants collected at the site found several additional PFAS, with PFOS being the most prominent. Suspect screening was then conducted on the formulations and provided several suspected PFAS in addition to the 24 targeted analyzed PFAS in 7 of the 10 samples, one of which showed no PFAS during targeted analysis. PFAA-precursor oxidation was then conducted on the two pesticide formulations with the greatest lists of suspected PFAS as validation of potential unknown PFAS in the formulations. This study revealed a previously unknown potential PFAS contamination source for rural and agricultural areas.

05.14.39 PFAS Exposure Assessment in Communities with Known Drinking Water Contamination

B. Goodwin, ATSDR / Office of Community Health and Hazard Assessment; R. Rogers, Agency for Toxic Substances and Disease Registry/Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry (CDC); P. Kowalski, K. Scruton, A. Pomales, Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry / Office of Community Health and Hazard Assessment

The Agency for Toxic Substances and Disease Registry (ATSDR) conducted biomonitoring exposure assessments (EAs) in communities near domestic military installations that have documented exposures to PFAS in drinking water. In all cases, drinking water concentrations of PFAS were mitigated prior to initiation of the biomonitoring efforts. ATSDR used a community sampling design at each site to determine the following: - The distribution of PFAS serum concentrations in communities with recent or past exposures to PFAS in drinking water - PFAS urine concentrations from a subset of participants with recent or past exposures to PFAS in drinking water - PFAS concentrations in indoor dust and tap water samples from a subset of homes of participants that provided biological samples All participants completed a questionnaire to gather information to characterize each person’s exposure. ATSDR shared individual serum and urine results with participants, and we shared community level results (geometric mean and 95^{th} percentile for each measured PFAS) publicly. Detailed analysis of the serum, urine, tap water, and dust samples is in progress to determine if associations exist between these results and responses to the exposure questionnaire. The following PFAS were present in serum concentrations above the national average (2015-2016 National Health and Nutrition Examination Survey): · PFOA (in 7 of 8 communities) · PFOS (in 7 of 8 communities) · PFHxS (in 8 of 8 communities) All drinking water samples collected during the EAs have had concentrations of PFOA + PFOS below EPA’s lifetime health advisory of 70 parts per trillion. ATSDR also evaluated associations between PFAS serum levels and exposure questionnaire results and will present these results for sites that have complete information at the time of the presentation. The findings and conclusions in this presentation have not been formally disseminated by the Agency for Toxic Substances and Disease Registry and should not be construed to represent any agency determination or policy.

05.14.40 Estimating Internal Exposure and Bioaccumulation Potential for Novel PFAS: A Tiered Modeling Strategy

C.A. Ng, M. Khazaee, M. Bedi, University of Pittsburgh / Civil and Environmental Engineering

The bioaccumulation potential of "legacy" long-chain perfluoroalkyl substances (PFAS) like PFOA and PFOS initially took environmental chemists and toxicologists by surprise because these chemicals behaved very differently than traditional lipophilic neutral organic compounds. Rather than accumulating in fatty tissues, perfluorinated acids were found to accumulate in liver and blood, and subsequent research identified binding to proteins and partitioning to membrane phospholipids as key drivers of the bioaccumulation of these chemicals. These findings revolutionized bioaccumulation and exposure assessment for PFAS. However, the perfluorinated acids on which these advances in understanding PFAS were based represent only a small proportion of the potentially thousands of structurally diverse fluorinated chemicals. Given the urgent need to fill critical data gaps on novel and emerging PFAS compounds with different structural features and key functional groups (e.g. cationic compounds, zwitterionic compounds, neutral/volatile compounds, cyclic compounds), the question arises whether the established understanding of PFAS structure-activity relationships will hold. This is of particular concern for understanding their bioaccumulation potential and tissue distribution, and for properly interpreting the results of toxicity assays. Here, we present a tiered approach to estimating the uptake and tissue distribution of structurally diverse PFAS compounds that relates their structures and physicochemical properties to three key biological phases: neutral lipids, phospholipids, and proteins. Using state-of-the-art structure-activity relationships for membrane-water and lipid-water partitioning as well as molecular modeling of protein-PFAS interactions, we classify a variety of PFAS structural subclasses according to their biological phase preferences and thereby provide a first-tier assessment of their tendency for bioaccumulation and likely tissue distribution. This approach can be used as a means to estimate internal dose from external exposure, to aid in interpretation of relative potencies across compounds.

05.14.41 Per- and Polyfluorinated Alkyl Substance Research Studies Conducted in a Heavily Compromised Zone

C. Powley, A. Redding, Calgon Carbon; S. Coleman-Kammula, Center for PFAS Solutions

The Center for PFAS Solutions is an analytical research laboratory located in an area of New Castle County, Delaware where the groundwater is contaminated with legacy per- and polyfluorinated alkyl substances (PFAS) that arise from heavy historical use of aqueous film-foaming foams (AFFFs) at the nearby Air National Guard base. The USEPA has designated multiple wells used to supply drinking water to approximately 8300 customers as having combined perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) levels well above the health advisory level (HAL) of 70 ppt. In addition, we have detected ppb levels of other long and short chain perfluorinated carboxylic acids and sulfonates in this water, as well as telomer sulfonates. All of these contaminants can be attributed to AFFF use, either as product components or as environmental degradants. The branched to linear isomer ratios suggest most of the contamination is 20 to 50 years old. Conducting ppt-level PFAS analysis in this environment is very challenging, and we will report some novel ways of monitoring and eliminating background contamination. One contaminant in particular is the 6:2 fluorotelomer sulfonate, which is ubiquitous in our laboratory atmosphere and can contribute to ng/mL background levels in water samples prepared using EPA Method 533. Other labs have reported this challenge as well, albeit at lower levels. We will report on method modifications we have made to eliminate this issue. In addition to supporting the local water companies, we are also conducting research studies to evaluate new and existing sorbents to determine their capability to remove the particularly troublesome short chain PFAS from well water, such as the perfluoro butanoic and pentanoic acids and sulfonates. One of these projects is a pilot-scale study currently underway near our facility, where different granular activated carbon (GAC) and ion exchange (IX) resins are being evaluated side-by-side using the water from the contaminated aquifer to evaluate their effectiveness. Both of these sorbents are effective for the commonly-monitored longer chain PFAS, with somewhat less affinity for the branched isomers. However, they appear to have a much lower relative affinity for the perfluorinated butanoic and pentanoic carboxlates PFBA and PFPeA.

05.14.43 Towards Improved Understanding of Environmental Impacts of Per- and Polyfluoroalkyl Substances in Recycling Streams

E. Bulson, A. Hicks, University of Wisconsin, Madison / Civil and Environmental Engineering; C. Remucal, University of Wisconsin Madison / Civil and Environmental Engineering/Environmental Chemistry and Technology

The environmental impacts of emerging contaminants per- and polyfluoroalkyl substances (PFAS) in recycling streams are not fully understood. In particular, there is a notable absence of information easily accessible pertaining to PFAS in metal-based recycling streams. However, several studies indicate that automotive shredder residue (ASR), the remaining nonmetallic fraction after shredded recyclable metals have been procured, as a potential source of PFAS. The British Metals Recycling Association (BMRA) reported perfluorooctanesulfonic acid (PFOS) concentrations ranging several orders of magnitude for vehicle seat covers (a component of ASR) when measured in parts per trillion (ppt), with the reported maximum concentration 54,000 ppt (2013). Furthermore, the New Hampshire Department of Health and Environmental Services (NHDES) reported that 67% of metal recycling facilities that participated in a statewide groundwater study had groundwater PFAS concentrations above ambient groundwater quality standards (2020). While the BMRA and NHDES data suggest potentially high PFAS concentrations in ASR along with leaching potential, available data is insufficient to quantify PFAS presence and mobility in the ASR waste stream. Furthermore, while it is anticipated that the PFAS content in ASR will decrease over time as a result of the EPA PFOA Stewardship Program, there is insufficient available data to validate significant PFAS decline within the ASR waste stream at this time. Equally important to quantifying PFAS in ASR is assessing the mobility of PFAS in the environment derived from ASR. Here we take a holistic approach to provide a broad overview of available data pertaining to PFAS presence in metal recycling systems and the evolving indications for potential exposures through recycling and disposal of PFAS-containing materials. We utilize this review of available information to identify knowledge gaps as they relate to PFAS in metal recycling streams.

05.14.44 PFAS in Sport Fish from San Francisco Bay

E. Miller, N. Buzby, R. Sutton, J.A. Davis, San Francisco Estuary Institute

The Regional Monitoring Program for Water Quality in San Francisco Bay has monitored per- and polyfluoroalkylated substances (PFAS) in sport fish since 2009. In 2019, the RMP increased the scope of PFAS chemical analysis, measuring 32 PFAS across five fish species collected at 13 locations, with PFAS detected in 14 of the 16 samples analyzed. Fish species were selected based on a number of criteria, including species that are popular for consumption, are sensitive indicators of problems (accumulating relatively high concentrations of contaminants), are widely distributed, represent different exposure pathways (benthic versus pelagic), and have been monitored in the past. Similar to previous years, perfluorooctanesulfonic acid (PFOS) was the dominant PFAS (averaging 62% of total PFAS concentration across all samples), followed by perfluorooctanesulfonamide (PFOSA; 7.4% of total PFAS) and perfluorododecanoic acid (PFDoA; 6.7% of total PFAS). In general, short-chain perfluoroalkyl substances (e.g., perfluorobutanoic acid or PFBA, and perfluorohexane sulfonate or PFHxS), perfluorooctanoic acid (PFOA), and most precursors were rarely detected, and most reported concentrations were close to detection limits. This study generated some of the first fish tissue monitoring data in species consumed by humans for newer PFAS such as GenX and ADONA in the US - though neither were detected. No human health or regulatory thresholds have yet been established for PFAS in San Francisco Bay fish. However, concentrations in Bay fish, particularly in the South Bay region, are persisting over time at levels that exceed thresholds that have been established by other states for development of consumption advisories. At the South Bay station, which has had the greatest and most consistent intensity of sampling over the three rounds, and where the contamination signal is relatively strong, the average concentration was higher in 2019 than in 2009 and 2014. The limited scope and inconsistency of PFAS sampling in sport fish over time (2009, 2014, and 2019) and changes in analytical methods prevent a rigorous analysis of temporal trends, but the available data indicate that PFAS contamination is persisting in the food web, with the Lower South Bay a region of particular concern.

05.14.45 A High-Resolution Reconstruction of PFAS Deposition in an Urban River Using a Radiometrically Dated Sediment Core

M. Cashman, U.S. Environmental Protection Agency / Atlantic Coastal Environmental Sciences Division (ACESD); A.R. Robuck, University of Rhode Island / Graduate School of Oceanography; T.B. Boving, University of Rhode Island / Department of Geosciences; M.G. Cantwell, U.S. Environmental Protection Agency

The use of per- and polyfluoroalkyl substances (PFAS) in industrial applications predates accessible analytical techniques for their identification in environmental matrices. Dated sediment cores offer a mechanism to determine past PFAS deposition and provide a better understanding of the fate of this complex class of contaminants in aquatic environments. In this study, a sediment core was collected from a dammed section of an urban river in Rhode Island, USA. The coring location was chosen for its proximity to former manufacturing facilities suspected to use PFAS. The core was radiometrically dated using ^{137}Cs and ^{210}Pb and analyzed for 24 PFAS compounds using a targeted analytical method. A modified Total Oxidizable Precursor (TOP) assay was then performed to identify presence of PFAS precursors. Suspect and non-targeted analysis was performed to identify additional PFAS missed in targeted analysis. Initial sediment concentrations showed temporal trends of PFAS preserved within the sediment record ranging from < 1-50 ng/g sediment. These data identify legacy PFAS compounds previously used in manufacturing processes over several decades, particularly long-chain perfluorinated carboxylic acids (PFCAs). This type of investigation provides a window into the behavior of PFAS once they enter the sedimentary environment.

05.14.46 Per- and Polyfluoroalkyl Substances (PFAS) in Breast Milk: Concerning Trends for Current-Use PFAS

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This is the first study in the last 15 years to analyze per- and polyfluoroalkyl substances (PFAS) in breast milk collected from mothers (n = 50) in the United States and our findings indicate that both legacy and current-use PFAS now contaminate breast milk, exposing nursing infants. Breast milk was analyzed for 39 PFAS, including 9 short-chain and 30 long-chain compounds, and 16 of these PFAS were detected in 4-100% of the samples. The total PFAS concentration in breast milk ranged from 103 to 1850 pg/mL with a median concentration of 121 pg/mL. Perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) were the most abundant PFAS in these samples (medians 30.4 and 13.9 pg/mL, respectively). Two short-chain PFAS, including perfluoro-*n*-hexanoic acid (PFHxA, C6) and perfluoro-*n*-heptanoic acid (PFHpA, C7), were detected in almost all of the samples with median concentrations of 9.69 and 6.10 pg/mL, respectively. Analysis of the available breast milk PFAS data from around the world over the period of 1996-2019 showed that while the levels of the phased-out PFOS and PFOA have been declining with halving times of 8.1 and 17 years, respectively, the detection frequencies of current-use short-chain PFAS have been increasing with a doubling time of 4.1 years.

05.14.47 Fine Powder PTFE: How Chemical, Physical and Biological Properties Affect Potential Exposures

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This work combines new and recently published data to expand our knowledge of per- and polyfluoroalkyl substances (PFAS) and inform efforts to protect public health. Data was developed to confirm that fluoropolymer polytetrafluoroethylene (PTFE, fine powder made without PFOA), will not partition to air, water or soil leading to potential inhalation, oral or dermal exposures, respectively, for biota or for the environment.Partitioning.t. Air: vapor pressure (very low, < 1 x 10^-10 mm Hg at 20 deg C), molecular weight, melting temperature OECD 102 (melt transition around 350 deg C, no further melting or decomposition below 400 deg C), thermal stability OECD 112 (no decomposition or chemical reaction observed < 150 deg C) thermal gravimetric analysis (5% weight loss observed at 549 deg C). These data confirm low inhalation exposure for biota.Partitioning.t. Water: water solubility, ready biodegradation OECD 301B (not biodegradable), inherent biodegradation OECD 302C (not biodegradable), toxicity characteristic leaching potential USEPA Method 1311 (passed). Data confirm low probability of water exposure to PTFE, degradants or leachables.Partitioning.t. Soil: toxicity characteristic leaching potential, molecular weight, soil adsorption, phototransformation on soil surfaces. High molecular weight and low leachables support lack of partitioning to soil.The stability, durability or persistence of fine powder PTFE enables “durable by design” products of high societal value (e.g., aerospace electronics, implanted medical devices, biopharma processing, mercury capture filters for coal plants, fuel cells, etc.). This OECD and EPA guideline data supports the stability of PTFE and the absence of leaching of or transformation to other PFAS, such as perfluoroalkyl acids (PFAAs). The high molecular weight and lack of oligomers or leachables < 1,000 Da renders PTFE not bioavailable or bioaccumulative and supports the lack of hazard of the polymer. Because this PTFE meets OECD Polymer of Low Concern criteria for particle size (>5 micrometers), it is not a nanoparticle.

05.14.48 The Spatial Pattern of Industrial Pollution and Water Quality Issues in Kentucky

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Water crisis is a global concern and the state of Kentucky (KY) is not an exemption, as residents struggle to access clean water, especially in some rural counties. Martin County, KY, being one of them, is significantly impacted. Especially after the spill in 2000, dumping about 1.16 billion of coal slurry in this region's water. Aside from Martin county, there are other counties within the state with contaminated surface water due to industrial pollution. Besides the benefits of an industrialized city, there are concerns about the environmental impact of these industries' emissions. Many industries' manufacturing processes allow the discharge of contaminants into the air and water. One of the pollutants of great concern is poly-fluoroalkyl substances (PFAs). PFAs are a class of highly fluorinated chemicals, persistent in the environment, and potentially toxic. In the United States (US), over six million people have been exposed to these chemicals through contact with contaminated drinking water. According to the International Agency for Research on Cancer (IARC), in 2016, some of these chemicals (PFAs and PFOS) were classified as group 2B; this means these chemicals are possible human carcinogens. This research focuses on industrial pollution and water quality issues in Kentucky (KY). The focus is on PFAs and some other industrial pollutants that affect drinking water quality. The data on toxic releases of industries in Kentucky was accessed and analyzed using Geographic Information System (GIS). The data is from the United States Environmental Protection Agency (EPA) Toxic Release Inventory program. Toxic releases of some chemicals such as PFAs and others that affect drinking water quality were of great concern. Some studies found that drinking waters with their sources from the Ohio River have a high concentration of PFAs; this research also looks into the proximity of some of these industries to the Ohio River and some other KY navigable waters and rivers as influent sources for water treatment plants. Results showed a possible relationship between the toxic releases of industries in KY and water quality issues, with GIS maps illustrating the proximity of some of these industries to the Ohio River, water treatment facilities, and households below the poverty level.

05.14.50 The Ecological Risk and Translocation of Poly- and Perfluoroalkyl Substances Within a Tidal Freshwater Potomac River Ecosystem

T. King, George Mason University / Environmental Science and Policy; T.B. Huff, George Mason University / SRIF; G. Foster, George Mason University / Chemistry and Biochemistry

With the development of new federal and state PFAS discharge and drinking water regulations, it is essential to understand the baseline environmental concentrations of PFAS through location-specific field assessments. Field measurements provide an opportunity to concurrently test PFAS bioaccumulation models of aquatic organisms, which have been shown to deviate from traditional indices such as the octanol-water partition coefficient. Bioaccumulation, bioconcentration, biomagnification, and trophic magnification factors have been proposed as alternative metrics with increasing levels of certainty, respectively. The study location of focus, the tidal freshwater Potomac River, provides hundreds of millions of gallons of drinking water for the Washington, D.C. area per day. Gunston Cove and Hunting Creek are two river embayments off the main channel in proximity to several potential PFAS point sources (e.g., wastewater treatment plants, military installations, and airports). Over the past few decades, each study location has transitioned from having phytoplankton-dominated to submersed aquatic vegetation (SAV)- dominated primary production, creating unique environmental conditions for the study of PFAS uptake through aquatic food webs. This study assesses the PFAS presence, bioaccumulation potential, and preliminary source characterization at two locations not known to be affected by incidental PFAS discharges. From May to September 2019, surface water, sediment, SAV, zooplankton, and banded killifish (*Fundulus diaphanus*) were collected from Gunston Cove and Hunting Creek. Solid phase extraction and micro-QuEChERS (Quick-Easy-Cheap-Effective-Rugged-Safe) methods were used to prepare sample media for PFAS trace level analysis on a liquid chromatograph triple quadrupole mass spectrometer (LC-MS/MS). A total of 28 PFAS were targeted, including perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkane sulfonates (PFSAs), and their precursors and alternatives. Preliminary results indicate the presence of six PFAS in surface water from both study locations at concentrations ranging from 1.5 – 90.9 ng/L. The findings of this study can advance the understanding of PFAS environmental baseline concentrations, extraction methodology for biological matrices, bioaccumulation potential in lower trophic level species, and preliminary source characterization.

05.15 When Standardized Regulatory Tests Are Not Enough

05.15.01 Addressing Challenges of Incorporating Higher Tier Data Into Ecological Risk Assessments for Crop Protection Products to Reduce Uncertainty

S. Levine, Bayer AG - Crop Science Division / Regulatory

Although higher tier methodologies typically provide data to address the assumptions and simplifications inherent in lower tier risk assessments, the design, conduct, and acceptance and application of higher tier approaches can pose specific challenges. The nature and scope of the studies that could be performed will depend on several factors, including the results of lower tier studies, environmental protection goals, related assessment endpoints, the environmental fate of the pesticide, and the use pattern of the pesticide. Tiered testing and assessment schemes have been developed to be a flexible framework to address changing testing and assessment needs and new hypotheses that may arise during problem formulation or as the outcome of lower tier testing. Therefore, a tiered framework offers many advantages in terms of supporting assessments that examine potential effects of pesticides. A common goal among data generators, sponsors, reviewers, and users of the data should ideally be to design an appropriate higher tier study to satisfy the needs of the risk assessor or risk manager within the bounds of current capability and without imposing unnecessary regulatory burden on registrants. An advantage for conducting some higher tier studies is that endpoints can be more relevant to actual environmental exposure, if properly designed, because factors that influence fate and exposure can be incorporated directly into the test system. Consequently, such studies should provide more confidence when predicting actual effects in the environment. In addition, tests can be designed to assess effects for a specific taxon that may be at risk, and post-exposure recovery can be assessed for both individuals and populations. However, there are challenges to conducting higher tier tests. For example, standardized higher tier methodologies are generally not available, and there can be disagreements over the relevance of different test designs and interpretation of outcomes. This presentation will provide several examples of higher tier and bespoke studies that have been used to assess specific protection goals, lower uncertainty, and demonstrate low risk. Examples will be drawn from studies with traditional agrochemicals and for insect protected genetically modified crops. In addition, recommendations will be presented on how to successfully overcome the typical challenges of incorporating higher tier data into ecological risk assessments.

05.15.02 Bifenthrin Is Not Bioaccumulative in Fish: Results from a Biomonitoring Program in Dosed Ponds in the Northern United States

D.R. Moore, C. Priest, Intrinsik Ltd. / Pesticides Group; G. Jones, Stone Environmental, Inc.; T. Geil, FMC Corporation; A. Samel, R. Trevisan, FMC Corporation / Environmental Sciences

In 2017, the Pest Management Regulatory Agency (PMRA) in Canada determined that the insecticide, bifenthrin, met the criterion of a bioaccumulative substance under the Toxic Substances Management Policy (TSMP), i.e., Bioaccumulation Factor (BAF) in fish > 5000 L/kg ww. The supporting evidence for this conclusion, however, was primarily from a field study that suffered from several major design flaws. In response to the PMRA designation, we undertook a rigorously designed field study to determine BAFs for bifenthrin in fish in two natural ponds in Michigan and North Dakota. Each pond was dosed with CAPTURE® 240EC insecticide (240 g bifenthrin/L) in late spring/early summer in 2018 and again in 2019. The ponds had been stocked with benthic and pelagic fish species prior to the initial application. Each year, water, sediment and fish samples were collected at regular intervals from the day before each application until the ponds froze over. Following chemical analyses, statistical analyses were carried out to determine concentration trends over time in each matrix in each pond. The analyses indicated that steady state or approximate steady state conditions generally occurred approximately 60 days after application (DAA+60), DAA+90, or later. We calculated BAFs for each year, each pond, and each fish species using three different methods: · The geometric mean fish tissue concentration was divided by the geometric mean water concentration for sample data collected from DAA+60 to the last sampling period of each year. · The geometric mean fish tissue concentration was divided by the geometric mean water concentration for sample data collected from DAA+90 to the last sampling period of the year. · The steady state fish tissue concentration estimated via segmented regression analysis was divided by the corresponding steady state water concentration. The estimated BAFs were well below the TSMP bioaccumulation criterion irrespective of calculation method. Within each combination of year, pond and fish species, the estimated BAFs using the three calculation methods produced similar values, increasing our confidence that the BAF values for bifenthrin do not exceed 5000 L/kg ww. The study results and statistical analyses demonstrated that bifenthrin does not bioaccumulate to levels in fish that exceed the TSMP bioaccumulation criterion. Therefore, bifenthrin does not trigger classification under the TSMP for bioaccumulation.

05.15.03 Weight of Evidence Assessment to Confirm the Low Bioaccumulation Potential of Bifenthrin as Defined Under Canada's Toxic Substances Management Policy

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The purpose of this research was to assess the bioaccumulation potential of the pyrethroid insecticide, bifenthrin, against the bioaccumulation criterion listed in the Canadian *Toxic Substances Management Policy* (TSMP). The bioaccumulation assessment was carried out using a weight-of-evidence (WoE) approach that considered the quality and results of aquatic laboratory, mesocosm and field studies, refined bioaccumulation modeling, and a comparison of the bioaccumulation potential of bifenthrin to other pyrethroids. The WoE assessment was carried out as prescribed by the OECD Series on Testing and Assessment No. 311 guidance. To be considered bioaccumulative under the TSMP, the bioconcentration factor (BCF) or bioaccumulation factor (BAF) calculated for the compound must exceed 5000 L/kg wet weight (ww). The estimated bioconcentration factors (BCFs) from the most reliable regulatory laboratory bioconcentration studies with fish and bifenthrin ranged from 1030 to 1414 L/kg ww and BAFs from reliable mesocosm and field studies were well below 5000 for fish. Mass balance food web bioaccumulation modeling conducted under different exposure conditions, not calibrated to the measured results of the field study, generated predicted BAFs for fish very close to the field measured values and were well below the TSMP criterion of 5000. Laboratory bioconcentration, modeling and depuration studies with fish were also examined for 5-7 other pyrethroids. As a class, BCFs, whether measured or predicted, were infrequently above 1000 and never exceeded the TSMP criterion of 5000. High-quality studies have demonstrated that pyrethroids, including bifenthrin, are rapidly metabolized and detoxified in organisms, which is the primary reason for their low bioaccumulation potential. The four lines of evidence, including laboratory data, mesocosm and field data, mass balance food web modeling and information from other pyrethroids, all support the conclusion that bifenthrin is not bioaccumulative as defined under the TSMP. Because the confidence in each line of evidence is high and the different approaches corroborate one another, we have high confidence in the conclusion.

05.15.04 Application of Mass Balance Models to Corroborate Empirical Bifenthrin Bioaccumulation Data

J.M. Armitage, AES Armitage Environmental Sciences, Inc; J.A. Arnot, ARC Arnot Research & Consulting / Department of Physical & Environmental Science/Department of Pharmacology and Toxicology; A. Samel, R. Trevisan, FMC Corporation / Environmental Sciences; T. Geil, FMC Corporation

Bioaccumulation assessments of organic chemicals can be conducted by considering endpoints from laboratory studies and more complex field studies. In some cases, supporting information describing study conditions and other influential parameters may not be available, making interpretation of empirical data challenging and uncertain. The objective of this work was to use mass balance bioaccumulation models to corroborate independent measurements of the bioaccumulation potential of bifenthrin in fish. A bioaccumulation model (AQUAWEB) was parameterized to simulate bifenthrin bioaccumulation under different exposure conditions, namely: (i) laboratory bioconcentration factor (BCF) tests for fish, (ii) a laboratory biomagnification factor (BMF) test for fish, and (iii) two field studies from representative geographical locations in the United States (Michigan, North Dakota). This analysis includes the synthesis of information from laboratory studies for bioaccumulation of bifenthrin in various aquatic species including algae, invertebrates and fish, as well as quantitative structure activity relationships (QSARs) validated following OECD guidance for application in regulatory decision-making contexts. The model predictions for lab BCFs, lab BMFs, fish concentrations in the natural environment, and field BAFs are based on measured ambient concentrations in the respective exposure scenarios (e.g., water concentrations in the lab BCF tests). Model-simulated BCFs were in agreement with the empirical data for the four data points considered (i.e., within ±30%) and all below a value of 2000 L/kg wet weight. The modeled laboratory BMF (0.1 kg lipid/kg lipid) also agreed with the empirical laboratory BMF data and both are below assessment criteria proposed in the scientific literature. Modeled field BAFs for the Michigan and North Dakota pond studies were generally within a factor of two of the empirical steady-state BAFs for the fish species and years with sufficient data to arrive at robust estimates. All credible empirical and *in silico* field BAF estimates were also below a value of 2000 L/kg. Collectively, the good agreement between the various measurements (lab BCFs, lab BMFs, field BAFs) and the model simulations provides a convincing body of knowledge (weight of evidence) characterizing the bioaccumulation potential of bifenthrin in aquatic ecosystems.

05.15.05 Toxicokinetic-Toxicodynamic Models as New Tools for Environmental Risk Assessment

S. Charles, University Lyon / Laboratory of Biometry and Evolutionnary Biology

Today, Environmental Risk Assessment (ERA) for chemicals is based on fitting standard dose-response (DR) models to quantitative data. Such data are usually collected from standard toxicity tests from which the concentration leading to 50% lethality or effect (LC50 or EC50) is usually estimated at the end of the exposure time. Such an evaluation implies that endpoints monitored over time are not fully exploited. Standard DR models also assume that the exposure concentration remains constant all along the experiment, what makes it impossible to extrapolate results to more realistic scenarios, such as time-variable exposure profiles. To overcome this gap, the use of the toxicokinetics-toxicodynamics (TKTD) models is today recommended, TKTD models describing effects of substances by accounting for the dynamics of exposure. In addition, TKTD models have many advantages in terms of mechanistic understanding of the chemical mode of action, of deriving time-independent parameters, of interpreting time-varying exposure and of making predictions for untested and time-variable exposure. Another advantage of TKTD models for ERA is that they make it possible to calculate any LCx,t or ECx,t for a chosen effect strength x and any given exposure duration t. Nevertheless, being based on differential equations, their mathematical complexity requires numerically integrations when fitting the model to data, so that in practice TKTD models are still not used very often. In order to allow more users to use TKTD models for regulatory risk assessment without suffering any technicalities, the availability of a software environment for an easy handling of TKTD models would be of great value. That is the aim of the R package 'morse' that is web interfaced within the all-in-one facility MOSAIC (). In this presentation, we will give firstly an overview of TKTD models with a focus on the General Unified Threshold model for Survival (GUTS). Secondly, handling GUTS models within R or MOSAIC will be illustrated with one example dataset. Lastly, the added-value of GUTS models for ERA will be discussed based on a collection of different datasets from which several calculations of the LC50 will be compared.

05.15.06 A Novel Method for Evaluating Toxicity of Pesticide-Laden Particulate Matter to Pollinators

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Recent data has revealed significant amounts of particulate matter (PM) containing multiple agrochemicals suspended in air around beef cattle feed yards in the Southern High Plains (SHP) region. Among the agrochemicals detected in PM, pesticides of different classes (e.g., neonicotinoids, pyrethroids, macrocyclic lactones) occur in relatively high concentrations. Acute toxicity values for many of the chemicals detected in PM have been established for a limited number of pollinators such as honeybees or mason bees, but little toxicity data exists regarding mixtures of these chemicals. This previously uncharacterized source of potential pesticide exposure among pollinators warranted further investigation. Thus, *in situ* studies conducted near PM sources offer valuable insight regarding biological uptake of agrochemicals transported via PM and subsequent effects on local pollinators. However, many potentially confounding factors may impact these types of field studies. Atmospheric conditions such as precipitation, wind direction and speed, and temperature; variation in pesticide application with season, year, and location; and other sources of potential pesticide exposure can contribute uncertainty and error to assessments of risk focused on pollinators embedded in these landscapes. We developed a laboratory-based closed system for circulating known amounts of aerosolized PM . This system houses pollinators (e.g., honeybees or mason bees) in cages that ensure constant exposure to the circulated PM. Clean PM – material similar in composition to PM found near feedyards but lacking any agrochemicals – can be added to the system and circulated for known durations. Further, PM fortified with a single agrochemical or mixture of chemicals at any desired concentration can be circulated in the system. These methods allow for controlled toxicity tests with agrochemical-laden PM effects on pollinators.

05.16 Environmental Risk Assessment

05.16.01 A Comprehensive Petrochemical Vulnerability Index for Assessing Relative Vulnerability of Marine Fishes in the Gulf of Mexico

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In the Gulf of Mexico, crude oil spills are a significant threat to biodiversity. Crude oil is toxic to Gulf marine species, and sudden, massive spills can result in irreparable damage to Gulf ecosystems. Currently, there is a lack of efficient mechanisms to evaluate biological and ecological impacts to Gulf marine species post-spill for individual and population-level impact assessments, resource allocation, etc., given the dearth of species-specific toxicological information for most Gulf marine species. A solution is a comprehensive petrochemical vulnerability index, composed of selected, scored trait-based indicators to quantify species' relative vulnerabilities to stressors, i.e., an oil spill. A multi-taxonomic petrochemical vulnerability framework for marine vertebrates and select invertebrates was recently developed by 28 international experts in species' petrochemicals sensitivity, marine ecology, behavior, taxonomy, pharmacokinetic modeling, molecular mechanisms, and petrochemical environmental chemistry. This theoretical framework has now been applied to the complete list of 1,670 bony and cartilaginous fishes present in the Gulf of Mexico, quantifying Gulf marine fishes' likelihood of exposure, species sensitivity, and population resilience to determine the fishes' relative vulnerability to oil spills. The most vulnerable Gulf fishes were identified, with all fishes classified categorically by low, moderate, and high vulnerability, and results were analyzed and assessed for initial validation. This vulnerability index of Gulf of Mexico fish species will facilitate rapid, effective action for conservation of species in the event of oil spills and will also prioritize species and areas for preventative mitigation efforts and targeted recovery from past spills.

05.16.02 A Risk Assessment of Pb Exposure for Wild Turkeys from the Big River Watershed in Southeast Missouri: Examining the Potential Health Impact for Wildlife and Consumers

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Lead (Pb) mining in the Big River watershed in southeast Missouri (SEMO) began in 1740 with the greatest production occurring during the early to mid-20th century. Due to elevated concentrations of Pb in water, sediment, and fish tissues, the Big River was designated as a Superfund site in 1992. Wild turkeys have been described as a potential receptor for toxic effects because they can be exposed to Pb contaminated soils through inadvertent ingestion during feeding and as grit. Turkeys are also a popular game species for Missourians and there is concern that elevated Pb in tissues could be a health concern for consumers. Gizzard content (grit), feathers, liver, kidney, and muscle (breast and thigh) were collected from male hunter-harvested turkeys to determine whether turkeys accumulate lead and other heavy metals through ingestion of Pb-contaminated soils, and whether tissue concentrations exceeded adverse health thresholds. Tissular concentrations of heavy metals from turkeys harvested in SEMO (n=22) were compared to tissular concentrations in turkeys harvested in uncontaminated regions in northeast and central Missouri (reference, n=15). The size of rocks (grit) from the gizzards of 7 individuals were also examined to determine whether turkeys select rocks consistent with the size of chat fragments in the Big River watershed. For all tissues, Pb concentrations were greater in turkeys from SEMO than at reference sites. Elevated concentrations of Pb in grit of turkeys in SEMO (25.1 + 42.7 ppm dw) was largely driven by 4 individuals with grit Pb concentrations ranging 23-124 ppm dw. The majority (81%) of grit was the size of fine/very fine gravel (range, 2.8-6.7 mm) which is similar to the size of chat on gravel bars in the Big River. Pb concentrations in bone of 50% of SEMO turkeys exceeded 20 ppm dw (range, 0.6 - 871 ppm dw) suggestive of excessive lead exposure (Franson and Pain 2011). Kidney Pb concentrations exceeded thresholds for subclinical lead poisoning (>2 ppm ww) in 20% of SEMO turkeys (kidney Pb range 0.03 – 4.5 ppm ww). Concentrations of Pb in muscles were similar between breast and thigh (0.037.6 ± 0.034.7 ppm ww) and below consumption advisory thresholds (< 0.30 ppm ww) for the majority of individuals examined. Elevated Pb concentrations (>0.9 ppm ww) in the breast muscle of 3 turkeys was suspected to be due to use of Pb shot by hunters. Log normalized comparisons suggested that Pb concentrations in body feathers could be used as a non-invasive indicator of Pb concentration in muscles and organs. Although the muscle of turkeys harvested adjacent to the Big River are safe to consume, turkeys may be experiencing adverse physiological responses to exposure to Pb contaminated soils. Ongoing analyses will examine harvest locations in relation to the Big River floodplain, and the relationships among Pb concentration, organ mass, and histopathology.

05.16.03 Adding the Chemical Environment into Environmental Burden Indices

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Recent studies have attempted to relate a person's environment to adverse health outcomes through a quantitative variable known as an environmental burden index. To date, the indices used have focused on socio-economic and demographic variables, such as poverty, income, food accessibility, and race, rather than on any proxies related to chemical contamination. We contend that an environmental burden index that incorporates specific variables related to chemical contaminants will outperform indices that are based solely upon socio-economic and demographic variables. To this point, we conducted two sets of analyses using pre-existing datasets relative to proximity to agricultural pesticides. In the first experiment, we analyzed the association between pediatric cancer incidence and several potentially carcinogenic pesticides and metals across the state of Idaho. The metal data were water concentrations from groundwater wells, obtained from the Idaho Department of Water Resources, whereas the pesticide data were county usage data obtained from the United States Geographical Survey (USGS). Results suggest that the pesticides metam, glyphosate, and di-chloropropene, derived using principal component analysis, explained a significant amount of variation in pediatric cancer across the state. In the second experiment, the cancer incidence data from 11 contiguous states in the Western U.S. was associated with pesticide data obtained from the USGS across the region at county resolution. Findings indicated a statistically significant association (P-value < 0.01) between fumigant mass, specifically the medium and high tertials of fumigant mass (33 and 66 percentile mass respectively), and cancer incidence across the region. While the data used in these models was course and indirect, the results are compelling. They suggest a more detailed analysis, including toxicity data from Tox21 (Toxicology Testing in the 21st century), could be particularly valuable. As evidenced by the experiment results of this study, incorporating the toxicological data into the environmental burden indices is likely to enhance its overall explanatory power for adverse health outcomes such as cancer incidence.

05.16.04 An Approach for Assessing Aquatic Plants Under the Toxic Substances Control Act (TSCA)

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In conducting risk evaluations under TSCA, EPA's OPPT accounted for sources of uncertainty in inter- and intra-species variation and laboratory to field extrapolation through the application of fixed assessment factors (AFs). AFs were applied to available ecotoxicity data to calculate concentrations of concern (COCs). COC = toxicity value / AF EPA/OPPT used an AF of 4 for acute algae endpoints and 10 for chronic algae endpoints. With the publication and peer review of the first 10 Risk Evaluations under TSCA section 6, EPA/OPPT received comments from peer reviewers on its approach for assessing aquatic plants, use of AFs, and quantifying uncertainty. To address comments, EPA/OPPT first conducted a literature search to identify methods used for assessing aquatic plants and new approaches for calculating AFs. Next, EPAOPPT used data on algae and aquatic plants from the ECOTOXicology knowledgebase, which includes endpoints for over 2,000 chemicals and 760 species, to explore novel methods to calculate data-derived AFs. The results of the analysis showed that for small datasets, due to high variation in toxicity observed across algae species, an AF of 10 is not protective of 95% of algae species. Therefore, EPA/OPPT identified a need to either increase the AF or the number of species represented in the dataset for chemicals being assessed. In the method described herein, EPA/OPPT provides recommendations for (1) how other lines of evidence may be used to provide data for additional species, and (2) how updated AFs may be used for small datasets. Further, in cases where a chemical has data on about eight or more aquatic plant species, EPA/OPPT recommends accounting for uncertainties using a probabilistic approach. In this proposed approach, a species sensitivity distribution (SSD) and a model averaged HC_{05} (i.e., the hazardous concentration predicted to be protective of 95% of species) are generated from a given toxicity dataset. Inter-species variation and model uncertainty are subsequently accounted for by using the lower 95% confidence limit of the HC_{05}, and additional uncertainties can be accounted for with the application of an AF of 1–5. 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.

05.16.05 Assessing Potential Ecotoxicological Impacts Due to Residential Pesticide Use

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While environmental impact studies on agrochemical use in commercial settings are common, very little is known about the impacts of residential use of these compounds. Several articles indicated that residential application of pesticides may be harmful to the environment. Consequently, assessment of ecotoxicological impacts of pesticides in a residential setting is essential. The Environmental Protection Agency (EPA) has developed and used several modeling tools to estimate the impacts of pesticides on several ecological receptors, including non-target plants, honeybees, herpetofauna (amphibians and reptiles), birds, and mammals. To the best of our knowledge, these models have not been applied to risk assessment of residential pesticide use; however, they are a logical first step. Thus, the current study describes the application of EPA models to conduct ecological hazard screening assessment of commonly used pesticides in residential settings based on information acquired via surveys. The study ultimately aims to improve the understanding of the potential ecotoxicological impacts associated with residential pesticide use.

05.16.06 Assessing Water Quality and Sediment Toxicity of the Middle White River Sub-watersheds, Arkansas, USA

R. DeRoin, J.L. Bouldin, Arkansas State University / Biological Sciences

Surface water is under increasing threats of pollution such as industrial and agricultural sources. Surface water is essential for domestic, agricultural, and industrial uses. In Arkansas, the Middle White River Watershed is on the 303d list established through the Clean Water Act. The 303d is a list of impaired and threatened waterways including what each waterway is impaired with. This section of the act was established as a way for the EPA and Congress to monitor the status of each body of water, and allows for each state to verify if the quality standards for its intended use are met. The Middle White River Watershed is impaired with turbidity, dissolved oxygen, pathogens, and total suspended solids. The watershed is used year-round for recreational and commercial activities. The surrounding land is primarily forests and pastures. Row crops dramatically increased from 1% in 2004 to 5.4% in 2006 in this area. The changing of the land use around the watershed is a potential source of impairment. Sub-watersheds play a crucial role in water health; however, it is not known how each contributes to the main watershed. The goal is to monitor four sub-watersheds of the Middle White River Watershed through the collection of water quality parameters that were performed during October 2019 through September 2021. Water collection at each sub-watershed site shows that the sites with pasture land cover have exceeded standards in turbidity and total suspended solids in the waterway than the urban forest sites. Based on the two sites exceeding the standard, I hypothesize that those site sediment toxicity levels have potential adverse effects on the benthic organisms. These results suggest that the change in land cover affects the impairment of the Middle White River Watershed, contributing to keeping this watershed on the 303(d) list of Arkansas.

05.16.09 Determination of Ecotoxicology Testing Needs Among Selected United States Federal Agencies

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United States (U.S.) regulatory and research agencies use ecotoxicity data to inform risk assessments and for other activities. Testing can encompass everything from soil microbes to entire field level studies, with many methods utilizing animals, including vertebrates. The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) identifies opportunities to develop non-animal alternatives to satisfy agency data and testing needs. The first step to developing alternatives to animal testing is identifying the specific tests that are conducted and the information used from those tests to inform regulatory decisions. Accordingly, the seven participating member agencies that comprise the ICCVAM Ecotoxicity Workgroup (EcoWG) (U.S. Department of Defense, U.S. Department of the Interior, U.S. Environmental Protection Agency, U.S. Food and Drug Administration, National Institute of Environmental Health Sciences, National Institute of Standards and Technology, and the U.S. Department of Agriculture): 1) developed a catalog of 95 standards, test guidelines, and guidance documents that are applicable to satisfy each member agency's needs, 2) described agency ecotoxicity data requirements, uses of the data, and the statutes that drive those needs, and 3) determined commonly used test species and endpoints with regard to ecotoxicity testing of animals. The EcoWG also identified flexibilities for using alternative methodologies, the information needed from alternative tests to fulfill the regulatory needs for whole animal ecotoxicity data, and whether data from non-animal alternative approaches are accepted to fulfill any of these regulatory needs. Finally, this review highlighted challenges and other considerations for the development of alternative methods and demonstrates the commitment by U.S. agencies to identify opportunities to implement alternatives to animal testing in appropriate contexts. This work was funded in whole or in part with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.

05.16.12 Innovating Microbial Pesticide Testing: An OECD Effort to Improve Test Guidelines

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There is an increased emphasis on expanding biodiversity and the sustainability of our agricultural production system by the agriculture industry, government regulators and other stakeholders. The desire for increased options for pest control, including biopesticides, creates an urgent need to improve the current regulatory approach, particularly for microbials, which constitute the largest category of commercially available biopesticides. While there have been many efforts to improve the hazard testing and regulatory risk assessment and approval process for microbials, the goal of a recent OECD effort is to drive toward an improved regulatory process for these products by improving the reliability and applicability of human and environmental hazard data that support microbial biopesticide registration applications, and their review and risk assessment. This effort intends to develop, propose, agree, and begin to implement a workplan to improve current test guidelines for microbial biopesticides and establish the framework and workplan for future test guidelines using New Approach Methodologies (NAMs) and / or other novel approaches for demonstrating a lack of hazard for microbial biopesticides. An overview of the project from an industry perspective will be provided in this presentation.

05.16.13 Integrated Assessment of Sediment Quality in Reservoirs and Lakes of the Saskatchewan River Basin

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The Saskatchewan River Delta (SRD) is the largest inland delta in North America. Over the past fifty years, anthropogenic changes upstream, including the construction of E.B. Campbell and Gardiner Dam, have altered the water and sediment transport dynamics such that a sediment deficit has been created. This has lowered the productivity of the SRD far below historical norms, resulting in cultural and economic devastation for the community of Cumberland House that is located in the SRD. Among multiple restoration options currently discussed, transporting sediment from elsewhere in the Saskatchewan River Basin to the SRD may be one potential solution. To screen candidate sediments for their quality, chemical and physico-chemical properties such as metal concentrations and sediment texture, as well as the potential to cause adverse biological effects, were assessed. Cell-based bioassays with either fish or mammalian cell lines were used to screen for cytotoxicity and detect the potential for effects from environmentally-relevant chemicals such as dioxin-like chemicals, estrogenic and anti-estrogenic chemicals, androgenic and anti-androgenic chemicals, as well as genotoxicants. Preliminary results suggest that some sediment from Codette Lake and Tobin Lake are not good candidates for remediation. This integrated sediment quality assessments will provide valuable information for future decision-making to the Delta Stewardship Committee in Cumberland House.

05.16.15 The MOLECULAR Toxicity of Microinjected Selenomethionine in Endangered, Common, and Model North American Fish

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Selenium (Se) is an atomic element and essential micronutrient for almost all forms of life with a distinctly narrow threshold between essential and toxic doses. It is also an important commodity and an incidental component of high-volume base resources (ex. Petroleum/coal), and multiple instances of ecosystem collapse have been observed as a result of accidental anthropogenic releases of excess Se to aquatic environments. While oviparous animals have shown the highest sensitivity overall, sensitivity differences are also found within classes of oviparous animals, and contemporary risk assessments of Se in aquatic environments are commonly complicated by a paucity of toxicity data within and across sensitive species, leaving some fish populations, such as the Se-sensitive white sturgeon (*Acipenser transmontanus*) of the Pacific North American coasts, at risk. This study was therefore designed to eliminate some of these unknowns. The specific purpose of this study was to observe the molecular, and associated apical, toxicities associated with microinjected, environmentally-relevant (3-45 µg total Se/g embryo dry mass), doses of selenomethionine (the naturally occurring organic form of Se most strongly implicated in Se toxicity in aquatic environments) in two model (fathead minnow: *Pimephales promelas*; rainbow trout; *Oncorhynchus mykiss*), and two novel (white sucker; *Catostomus commersonii*; white sturgeon; *Acipenser transmontanus*), environmentally-relevant fish species. Initial injection success was suggested by biological responses associated with SeMet toxicity across fish species (ex. craniofacial and spinal deformities, edema) and were later confirmed to be accurate using chemical analysis. Rainbow trout showed remarkable sensitivity to both the treatment and the injections, and nascent cross-species transcriptomic and proteomic analyses suggest that key oxidative-stress defense mechanisms (ex., superoxide dismutase and glutathione) underlie the chemical's toxic mechanisms. It is hoped these data will ultimately help to reveal the specific toxicity mechanisms and pathways predictive of adverse apical Se impacts within and across species to facilitate future Se risk assessments, and that they will also continue to contribute to the development of the EcoToxChip, a qPCR screening tool intended to expedite the risk assessment of chemicals produced in quantities of concern that currently lack toxicological characterization in aquatic environments.

05.16.16 Towards a Proactive Stance for Identifying Chemicals of Emerging Concern: The Department of Defense Proposed Dried Blood Spot Repository

L. Holden, M.S. Johnson, U.S. Army Public Health Center / Toxicology

One of the challenges of our time is monitoring exposures to new and emerging chemicals. This challenge is particularly relevant to military personnel who are often placed in potentially harmful situations where conventional means for measuring exposure are impractical. Relatively recent examples include exposures to burn pits, oil well fires, and combustion products from military-unique materials. The current practice of collecting environmental exposure samples provides only a site-specific snapshot in time. It ignores oral or dermal exposure routes and may not capture the person's location in that environment and what has been absorbed and metabolized. Dried blood spots are an exciting technology for application to chemical exposure biomonitoring efforts: they are inexpensive to collect and store, stable for years to decades, minimally invasive to collect, and do not require a cold chain. Dried blood spots are easily shipped domestically and internationally, and they capture the actual systemic concentration of relevance. In addition, there is an ever-expanding list of published analytical methods (2000+) applicable to dried blood spots. They have the added benefit of enabling the characterization of biomarkers of effect that are well calibrated to adverse pathologies by means of targeted or non-targeted (e.g., -omics) approaches, which may capture the peaks and continuity of exposure more effectively than passive sampling methods. Drawbacks of dried blood spots include the lack of biological exposure criteria for systemic chemical concentrations of emerging chemical hazards that may be measured in dried blood spots, and a limited capability to capture exposure to volatiles. However, dried blood spots provide a platform to collect and store data in a cost-effective way that allows us to integrate known or suspected exposures into both qualitative screening for hazards and quantitative assessment for disease association. A retrospective sample library of dried blood spots will aid in determining the etiology of future health effects due to both known and unknown chemical exposures.

05.16.18 Using the Chemical Aquatic Fate and Effects Database (CAFE), a Tool that Supports Assessments of Chemical and Oil Spills

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The Chemical Aquatic Fate and Effects (CAFE) database is a tool that facilitates assessments of accidental chemical and oil releases into aquatic environments. CAFE contains aquatic toxicity data summarized in the form of species sensitivity distributions (SSDs) with associated 5th percentile hazard concentrations (HCs). Since its initial release in 2015, CAFE has been used in hundreds of chemical and oil spills. However, for many chemicals and oils, gaps in species diversity and toxicity data limited the development of SSDs, which showed most prominently in emerging oils such as diluted bitumen and biodiesel. These emerging oils (~20 oils) will be included in the next version of Web CAFE in early 2022. In CAFE's upcoming 2022 web version, some of these data gaps were also addressed with Interspecies Correlation Estimation (ICE) models. The incorporation of ICE predicted values into CAFE allowed the development of >800 new SSDs and increased diversity in SSDs by an average of 34 species. With the addition of emerging oils and ICE models into CAFE's upcoming 2022 web version, more SSDs are generated, increasing CAFE's capacity to respond to chemical and oil spills. Attendees will be provided an overview of how CAFE SSDs are used to respond to chemical and oil spills. Diluted bitumen oil will be used as a case study oil to display available data and ICE model predictions.

05.20 Late Breaking Science: Environmental Risk Assessment

05.20.01 A Pilot Human Study: Altered Human Gut Microbiome Richness Is Associated to Manganese Exposure in Northern Italy

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Manganese (Mn), in trace amounts, is essential for human metabolism, central nerve system development, skeletal development, immune system and the antioxidant system. Mn exposure exceeding the physiologically required dose may adversely impact human health. It is possible that Mn impact health by disrupting the gut microbiome. Although several animal studies showed age and sex specific changes in gut microbiota by Mn exposure, there are limited reports on the effects of Mn on the human gut microbiome. We hypothesize that higher Mn exposure in humans would be linked with altered microbiome composition and community diversity. To test this hypothesis, In this pilot study, we used 16S next generation sequencing method to survey the gut microbiome composition and diversity among 100 young adults born and lived in three areas in Northern Italy (Bagnolo Mella (BM), Valcamonica(VC), and Garda Lake(GL)) that are characterized by different levels of ferromanganese alloy plant activity (currently active, historically active, reference with no activity, respectively). Using residence as a proxy for Mn exposure, we compared the microbial community richness among the three groups of subjects (33 BM, 43 VC, 24 GL) using a student-t test. We further compared the taxa abundance by non-parametric Wilcox rank test. Finally, we compared the overall microbial similarity measured by the Bray-Cutis distance matrices with a permutational multivariate analysis of variance approach([Adonis] in R). Results were FDR adjusted for multiple comparisons. Comparing to GL, our results show the samples from currently active exposed to Mn in BM area with much lower microbiome richness measured by observed taxa (mean =278 vs 317, p-value=0.014) and Shannon (mean =4.37 vs 4.58, p-value=0.055) (Figure 1A). However, we did not observe a significant shift in the overall microbiome structure (beta diversity visualized using Non-metric Multi-dimensional Scaling (NMDS) plot in Figure 1B) or the relative abundance of major taxa (Figure 1C, D) by the residence. Our results suggest a potential role of environmental Mn exposure on human health by reducing the gut microbiome community richness.

05.20.02 Bayesian Network Human Health Risk Assessment of Per- and Poly-Fluorinated Substances (PFAS) via Fish Consumption in Lake Worth, Texas

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The Texas Department of State Health Services (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. In 2020, DSHS staff sampled a total of 60 total fish from five species at 10 locations in Lake Worth, Texas. We analyzed the sampled fish tissues for 28 PFAS compounds and are using a Bayesian network as part our risk assessment. Bayesian networks are models that relate complex variables through conditional probabilities in a directed, acyclic graph. This approach allows us to calculate the probability of multiple adverse health effects occurring to multiple exposure groups, and to assess risk based on the full distribution of measured PFAS concentrations and demographic parameters instead of using representative means. This type of approach is also valuable for creating a structure to recognize and quantify uncertainties within the pathway, creating a framework that can easily incorporate future research, and inform management decisions such as issuing consumption advisories for fish species that present a health risk and initiating new research projects to address areas of uncertainty.

05.20.03 BEEHAVEecotox - a Mechanistic Effect Model for Honeybees

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Bees are important pollinators and thus an essential part of the environmental risk assessment of pesticides in the EU and in the US. Here, we introduce BEEHAVEecotox; an ecotoxicological model that mechanistically links exposure of bees in the field with the hazard profile for individual honeybees, leading to emerging colony effect. It is an addition to the widely used and extensively tested BEEHAVE colony model. The mechanistic link allows the translation of results from standard laboratory studies to relevant processes and parameters for simulating bee colony dynamics. The BEEHAVEecotox model includes 4 submodules: an external exposure module, in-hive fate module, water foraging module, and an effect module. The external exposure module incorporates the concentration of PPPs in the bee-relevant matrices such as nectar, pollen, and water. When foragers forage on these matrices, they receive an oral dose of the PPP, and they can be exposed via contact on the day of application. The water foraging module incorporates the need for water for cooling of the hive and dilution of stored honey, including potential exposure to PPPs. The in-hive fate module simulates the entry and mixing of the PPP into the hive, through nectar, pollen, or water. The effect module covers the mortality due to exposure for the different cohorts. It uses the slopes and LD50 values of standard acute contact, oral, chronic oral, and larvae studies as inputs. The BEEHAVEecotox model was validated against two semi-field studies with a tunnel setup with two PPPs with different modes of action (dimethoate and fenoxycarb). The validation showed that the BEEHAVEecotox model captured the initial effects on colony strength and the subsequent colony dynamics well for both substances. The model predicted the relative magnitude of effect at colony level directly after application, as well as the long-term reduction in colony strength in the post-exposure phase after the tunnel and the lack of recovery of the colony. The BEEHAVEecotox model is a suitable tool to predict the effects of PPPs on bees. It is the first model to mechanistically predict PPP exposure to foragers and within the hive from several different routes of exposure. For the regulatory risk assessment the model can potentially be used to extrapolate from laboratory to semi-field and field studies. Furthermore, it offers the possibility to study the effects in different crops and regions and to test different mitigation strategies.

05.20.04 Combining Ecological Risk Assessment and Life Cycle Indicators in a Tool to Support Eco-Innovation of Personal Care Products

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Companies manufacturing personal care products (PCPs) are increasingly interested in designing new products with improved environmental sustainability characteristics. Eco-innovation is an approach to product design that accounts for sustainability throughout the design process. Environmental assessments for products with down-the-drain disposition such as PCPs have traditionally used end-of-life ecological risk assessment (ERA) methods. More recently, regulators in Europe have proposed broad use of life cycle assessments (LCAs) to evaluate PCPs, which include different impact indicators than in ERAs. To support eco-innovation initiatives at the early design stage, a new spreadsheet tool was developed using methods from both the ERA and LCA paradigms. This tool considers all impacts commonly included in ERAs and LCAs that can be evaluated with data available at the early product design stage (i.e., before supply chains have been determined). The tool's output is a rank value between one (best) and ten (worst), representing the environmental safety profile of the product by integrating information for ten impact categories. Ecological effects, typically included in both ERA and LCA, are evaluated with the highest level of sophistication, given its known importance for down-the-drain products. Predicted no effect concentrations (PNECs) are automatically generated in the tool for water, soil, and sediment to evaluate the potential for ecological effects. These PNECs can be used separately in ERAs, which are sometimes required to substantiate "green" marketing claims. Additional impact indicators from ERAs considered include the potential for bioaccumulation, contamination of groundwater, and persistence in the environment. Included impact indicators common in LCA are eutrophication, photochemical ozone (smog) formation, mineral resource depletion, climate change due to direct greenhouse gas emissions, and stratospheric ozone depletion. A category for "other" effects is also included so newly discovered or unique concerns can be integrated. Indicators are aggregated using weighting factors determined by the end user, which account for scientific understanding and organizational priorities. The rank values calculated using the tool differentiate among formulas using the 10 environmental risk and sustainability impact indicators, providing valuable information to product development teams at early product design stage.

05.20.05 Emergent Adverse Outcome Pathways and Their Potential to Contribute Novel Toxicological Knowledge

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Adverse outcome pathways (AOPs) are used to encode structured toxicological knowledge. User-contributed online knowledgebases, such as the AOPWiki, contain AOPs that span diverse biological and toxicological domains. When AOPs are shared publicly, contributors are doing more than just disseminating knowledge of their specialties, they are also creating the potential for new knowledge through emergent AOPs across domains. Emergent AOPs result when key events are shared across user-defined AOPs. Recent research shows that emergent AOPs far outnumber user-defined AOPs in the AOPWiki. However, given the diversity of AOPs, emergent AOPs may provide novel toxicological insight, or they may be computational artifacts. Therefore, methods must be developed to assess their biological plausibility and prioritize them for further expert review. This paper demonstrates the usefulness of semantic network analysis of the user-defined and emergent AOPs for this purpose. Results show that unspecified emergent AOPs have, on average, comparable if not higher levels of semantic coherence as user-defined AOPs. These results indicate that emergent AOPs represent a large amount of valuable untapped AOP knowledge and reiterate the value of further enhancing user-contributed public knowledgebases, such as the AOPWiki and broader AOP knowledgebase. *The contents of this abstract neither constitute nor necessarily reflect USEPA policy.*

05.20.06 Relevance and Limitation of Integrative Passive Samplers in Support of Surface Waters Monitoring: Results of a French National-Scale Study

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The implementation of the European Water Framework Directive (WFD) requires the monitoring of contaminants in the aquatic environment to prevent any damages to both human health and ecosystems. In this context, over a hundred organic and inorganic substances have been selected as priority contaminants in France and the measurement of their occurrence levels is necessary to ensure that good water-quality standards are maintained. For 2 decades, integrative passive samplers (IPS) have been developed for the monitoring of organic and inorganic contaminants. In this context, the French government relied on the expertise of AQUAREF (national water reference laboratory in support of the WFD) to set up a national-scale study in order to demonstrate the relevance and limitations of IPS for the regulatory monitoring of contaminants in fresh and coastal marine waters. To achieve this objective, three complementary IPS allowing the sampling of inorganic and organic contaminants using both a spatial and a temporal sampling design. A total of 342 Polar Organic Chemical Integrative Samplers (POCIS), 106 Diffusive Gradients in Thin-films (DGT) and 229 Silicone Rubbers (SR) were deployed during two weeks on twenty sites spread throughout French rivers and coastal areas, including overseas territories (spatial approach), therefore covering sites located under various anthropogenic pressures (urban, industrial, agricultural). The temporal approach consisted of a total of 427 POCIS and 276 DGT deployed continuously during 1 year and retrieved every 2 weeks on 3 different sampling stations. In addition, spot samples were systematically collected during deployment and recovery of IPS. The relevance but also the drawbacks of these tools are discussed with regard to the operational aspects and the level of information on the chemical contamination of the water. The results confirm the benefit of implementing chemical monitoring by IPS, and demonstrate their applicability in the context of regulatory control networks. IPS allows lowering the limits of quantification increasing the frequency of quantification, which led to the detection of more molecules, and to lower uncertainties on the annual average concentration (AAs). Finally, the assessment of chemical status (comparison of AAs with EQS) based on AAs calculated using IPS is consistent with the one determined from spot sampling. Furthermore, the use of IPS allows to qualify the status of more sites than with spot sample.

05.20.07 Understanding the Interrelationships Between Chemical Contaminants, Harmful Algal Blooms, and Temperature: A Scoping Review

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Karenia brevis, a toxic dinoflagellate, mostly inhabits the Gulf of Mexico. The unchecked toxic blooms of *K. brevis* are predominantly associated with the Florida red tide. *K. brevis* produces a potent neurotoxin called brevetoxin. Brevetoxin, released in water, is known to cause morbidity in humans and mortality or morbidity in marine birds, mammals, and other marine organisms. Different physical, chemical, and biological factors contribute to blooms, and the influences of temperature changes and chemical nutrients on Florida red tide are an active area of research, as these are most amenable to environmental policy interventions. The purpose of this review was to summarize knowledge about the influence of temperature and chemical nutrients on the frequency and distribution of *K. brevis* blooms, and identify avenues of future research. The scoping review methodology framework by Arksey and O'Malley (2005) was followed for reporting this study. The study protocol is registered and available on Open Science Framework (OSF) at https://doi.org/10.17605/OSF.IO/DR8SF. Web of Science, Academic Search Complete, and PubMed databases were searched for English-language articles published between 2000 and 2021 using keywords. The keyword search in the databases yielded 2276 citations. After title/abstract and full-text screening, eight studies were included in the review. We found that temperature negatively affected the *K. brevis* bloom, both in the environment and under laboratory conditions, and nitrogen was crucial in persistent growth and maintenance of *K. brevis* bloom. However, phosphorus did not affect the *K. brevis* bloom abundance. The enormous growth of *K. brevis* bloom in the past 50 years can be explained by nutrients introduced into the water bodies due to human activities. We recommend improving data quality of nutrients in aquatic system and developing new strategies for collecting water samples to improve red tide forecasting, which will result in better understanding of the environmental factors driving red tide blooms.

05.20.08 Use of Simulation Methods to Predict Membrane-Water Partitioning and its Application Within Environmental Risk Assessment

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Within environmental risk assessment, octanol-water partitioning (log Kow) is commonly used as a physical-chemical descriptor for hydrophobic properties in aquatic toxicity predictions. Whilst log K_{ow} is generally considered acceptable for small, neutral organics, it is under increased scrutiny from both scientific and regulatory organisations with regards to its relevance in understanding the behaviour of ionisable compounds and surfactants. There is growing evidence that alternative partitioning parameters such as membrane-water partitioning (log *Kmw*) can provide a more biologically realistic approach to describing the interactions of charged, amphiphilic or surface-active compounds (e.g. perfluoralkyl substances and linear alky sulfates and sulfonates). Log *Kmw* also offers valuable new options for the development of New Approach Methodologies (NAMs) such as QSARs in support of a future-looking chemical safety agenda without the use of animals. However, accurate laboratory measurement of membrane-solute interactions is challenging and time-consuming. Moreover, membranes differ in composition and cholesterol content which affects the interactions with a given solute and can make experimental measurements more complex. Molecular dynamics simulations are an attractive alternative to experimental measurement, but they can be restrictively slow. Coarse-grained modelling is often utilised to study biological systems, as it decreases the degrees of freedom of the system, allowing for access to larger systems and timescales. Thus it can quickly provide accurate predictions of partitioning into multi-component systems, offering tangible opportunities for avoiding the complexities of experimentally derived values. Here we present a largely automated workflow for running coarse-grained simulations for neutral and ionisable molecules. The method uses freely available, open-source tools to parametrise a coarse-grained model of a solute, run a series of simulations, and analyse the results to obtain log *Kmw*. This poster will show how the coarse-graining approach can be successfully applied to a diverse range of molecules, including many which are difficult to model using other approaches, and apply these values in QSAR for predicting acute toxicity to aquatic species. We will also show that our simulations can be used to accurately model different membrane compositions and will therefore be useful for modelling partitioning in a range of biological contexts.

06.01 Mining: Innovative Approaches to Monitoring, Assessing and Remediating Contaminants of Concern at Mine Sites

06.01.01 Bioaccumulation and Speciation of As and Hg in Fish Tissue Within Se-Rich Aquatic Environments Near Sudbury, Ontario

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Mining activities potentially introduce a wide variety of trace elements to aquatic systems, even long after mining activities cease. These trace elements can interact with one another, altering their potential effects on biota. For example, selenium (Se) can have an antagonistic effect on mercury (Hg) and arsenic (As) bioaccumulation, two elements of particular concern in northern ecosystems. Furthermore, once incorporated into biota, these elements may enter biotransformation pathways that alter their chemical form, changing accumulation, excretion, and toxicity characteristics. This research will examine the accumulation and speciation patterns of As and Hg in freshwater fish around Sudbury, Ontario, Canada, a selenium-rich region. Speciation analyses will be performed using ion chromatography coupled to an inductively coupled plasma mass spectrometer (IC-ICP-MS); methods are currently being validated and samples are being collected. Lakes are being selected based on past studies and provincial monitoring data and will include systems with fish known to be high in total [Se], as well as systems without these flags. The goals of this research are to: (1) increase our understanding of trace element speciation patterns in Se-rich legacy mining environments; (2) help refine consumption guidelines, which are currently based on total As and Hg concentrations, a proportion of which is assumed to be in the most harmful form (i.e., methyl Hg and inorganic As); and (3) guide mine closure and monitoring plans in mining regions.

06.01.02 Insights into Mining Remediation: Microbiome Analysis of Soil and Rock Samples from Abandoned Lead-Silver Mines in the Arizona Sonoran Desert

D.A. Lucas, University of Arizona; T. Cahill, P. Marshall, Arizona State University / School of Mathematical and Natural Sciences

There are many former mines throughout the Sonoran Desert of Arizona left abandoned without environmental remediation. It was hypothesized that soils in mine waste rock piles would exhibit overall decreased microbial diversity compared to control samples due to the presence of toxic elements. It was additionally postulated that the unique environment within mine waste would allow certain bacteria to proliferate relative to undisturbed natural soil. Five former lead-silver mines were selected for study. Each mine was sampled in five different locations. Three control samples were collected for each mine nearby. Mineral samples of high-grade ore were also collected to look for chemolithotrophs. These mineral samples consisted of a galena core (PbS) with layers of alteration products. Elemental analysis of soil by x-ray fluorescence indicated varying concentrations of toxic elements between mine and control soils. Concentrations of Pb, As, and Cu were as high as 190, 561, and 30 times greater in mine soils than control soils, respectively. Interestingly, no arsenic was found in soils from one of the mines although natural concentrations were detected in the corresponding controls. Soil total DNA was extracted and analyzed via 16S rRNA sequencing and analysis. Of the 1,159 different bacterial species detected, 26% were conserved between all sample types, 50% between soils and their controls, 1.8% between soils and ore mineral samples, and 0.8% between controls and ore mineral samples. 27 (2.3% of total species) species were found only in minerals, 141 (12.2% of total species) only in mine soils, and 80 (6.9% of total species) found only in controls. This suggests that mining may modulate the soil microbiome to allow proliferation of bacteria not naturally occurring within desert soils while no longer supporting native species. Additionally, mining activity may increase the availability of minerals such that they become accessible to new bacteria like chemolithotrophs. Abundance was found to be decreased in mine soils compared to controls while microbial diversity was conserved. Both abundance and diversity were decreased in ore mineral samples compared to both soil sample types, but evenness was conserved between all sample types. These results suggest that while mining reduces the abundance of bacterial species in soil, it does not decrease the variety of species able to survive although changing the natural bacterial composition.

06.01.04 Modeling Future Changes in Element Concentrations of 99 Untreated Discharges from Legacy Mines in Japan by a Hierarchical Log-Linear Model

Y. Iwasaki, National Institute of Advanced Industrial Science and Technology / Research Institute of Science for Safety and Sustainability; K. Fukaya, National Institute for Environmental Studies; S. Fuchida, Waseda University / Faculty of Science and Engineering; S. Matsumoto, D. Araoka, National Institute of Advanced Industrial Science and Technology / Geological Survey of Japan; C. Tokoro, Waseda University / Faculty of Science and Engineering; T. Yasutaka, National Institute of Advanced Industrial Science and Technology / Geological Survey of Japan

Projecting future changes in the concentrations of trace metals in mine drainages, which can cause severe environmental impacts, is crucial to strategically optimize the treatment and management of such drainages. In this study, based on 17-year data for 99 untreated drainages from legacy mines in Japan, we developed a Bayesian hierarchical log-linear model that can capture temporal changes in the concentrations of seven elements (Cd, Pb, As, Cu, Zn, Fe, and Mn) in individual mine drainages. Results of the modeling showed that, during 2003–2019, although overall decreasing trends were observed for most elements across all the drainages evaluated, decreases in the concentrations of these elements were not evident in many mine drainages. In addition, any increase in the number of mine drainages with element concentrations below nationwide drainage standards over the next 100 years will likely be limited (e.g., approximately 10 drainages for Zn and Fe at median estimates). These results suggest that it is probably too optimistic to assume that the element concentrations of mine drainages will always decrease, or that these drainages will satisfy drainage standards (permits) in the not so distant future.

06.01.05 Development of Methodology for Early Life Stage Testing with Redside Shiners and Evaluation of Their Sensitivity to Maternally-Derived Selenium

B. Lo, Simon Fraser University; J. Van Geest, Golder Associates Inc.; J.R. Elphick, Nautilus Environmental Company, Inc.; M. Giorgini, M. Stokes, S. Weech, Minnow Environmental, Inc.; A.M. deBruyn, Golder Associates, Inc.; C. Good, Teck Coal Limited; M.C. Arnold, Duke University

The redside shiner (*Richardsonius balteus*) is a small-bodied cyprinid found in freshwater systems of northwestern North America. Elevated concentrations of selenium have been measured in ovaries of some fish of this species in the Koocanusa Reservior, downstream of metallurgical coal mining operations in southeastern British Columbia, which has led to concern regarding the potential for adverse effects occurring on this species. Adverse effects associated with selenium occur primarily in developing early life stages associated with offspring of exposed adults. Redside shiners have not been spawned and reared before in the laboratory, therefore methods for capture, spawning, fertilization and laboratory rearing were developed and used to assess the effects of maternally-derived selenium on development of offspring. The results of this study demonstrated that this species could be successfully spawned in the field and reared in the laboratory with a high rate of survival. There was no adverse response on survival or incidence of deformities related to selenium exposure in the developing fish at selenium concentrations of up to 28 mg/kg dw in the egg. Concentrations of selenium in the developed eggs were approximately 50% lower than in the residual ovary, on average, demonstrating that egg selenium declined during final maturation of the egg.

06.01.06 Novel Technologies Applied to Reduce Selenium and Nitrate at Mine Sites

K.M. Bechard, S. Mancini, Geosyntec Consultants, Inc.

Elevated selenium and nitrate concentrations in water leached from mined-rock piles are of significant concern for mine sites. This presentation will provide a brief overview of the technologies historically used to remove or reduce these compounds as well as go through a couple of case studies based on current pilot systems which use novel passive and semi-passive treatment designs. The novel case study designs harness the capabilities of specific bacteria to aid in the reduction/ precipitation of these constituents, reducing their concentrations by orders of magnitude in some cases. Input and output concentrations will be shared along with key design optimization considerations.

06.01.07 Application of Quantitative Mineralogy to Determine Sources of Airborne Particles at a European Copper Smelter

M. Kelvin, Queens University/XPS; Y. Gopalapillai, International Copper Association, Ltd. / Material Stewardship Program; S. Verpaele, Belgian Centre for Occupational Hygiene; M. Leybourne, D. Layton Matthews, Queens University / Geology

Activities performed at mineral processing operations such as mills, smelters and refineries, are capable of producing significant quantities of airborne particles, which can contain potentially hazardous metals. To ensure that regulatory compliance is maintained, worker exposure is regularly monitored. Determining the quantity and type of airborne particles present permits the operation to identify the sources of dust, implement proper dust suppressant strategies, and ultimately, limit worker exposure. Conventional methods of analysis, such as chemical assay, are unable to rigorously differentiate between phases containing the same elements and may result in ambiguity related to the source identification of the airborne dust. A combination of Quantitative Evaluation of Materials by Scanning Electron Microscope (QEMSCAN) and chemical characterization has been used to evaluate the airborne dust collected by using personal monitoring devices stationed at key locations throughout a Cu smelter in Europe. Additional mineralogical and geochemical analysis of bulk materials that were present at the operation area offered a direct comparison between the airborne dust and their possible sources. The Cu phases present in the airborne dust are reflective of the activities performed at specific locations. Near the anode and electric furnace, the majority of Cu in the airborne dust is carried in metals and oxides (60-70%), and in the batch preparation area where Cu concentrate is received, significant amounts of Cu are carried in sulfide minerals (chalcocite, chalcopyrite/bornite, 50-70%). The distribution of Cu phases at the individual locations is comparable between the inhalable and respirable fractions sampled. The results of the analysis will help identify the origin of emissions specific to location and activity, assist personnel in developing the appropriate mitigation strategies to limit worker exposure, and further understanding of the health risks associated with exposure

06.01.08 Environmental DNA (eDNA) Metabarcoding as a Risk Assessment Tool for Mining-Impacted Streams

J. Feller, Ohio State University / Department of Evolution, Ecology, and Organismal Biology; K. O'Reilly, Ohio State University / Department of Environmental and Natural Resources; C. Kinney, Ohio Division of Natural Resources; S.F. Spear, The Wilds Conservation Center; R.P. Lanno, Ohio State University / Department of Evolution, Ecology, and Organismal Biology

There is a pressing need for new biomonitoring methods in environmental risk assessment that can measure local biodiversity 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 multiple species present in an area. To test the effectiveness of this new technology in applied settings, eDNA was collected from various stream locations across southeastern Ohio that are impacted by acid-mine drainage from either historical or active coal-mining operations. These locations are routinely surveyed by the Ohio Division of Natural Resources (ODNR) for fish and macroinvertebrates via electrofishing and kick-net sampling by and at all sites water quality parameters (pH, metals, conductivity) were assessed. The goal of this proof-of-principle study is to compare biotic indices calculated by ODNR surveys to data generated by eDNA metabarcoding. eDNA samples were collected across 9 different sites and at 3 sites eDNA was sampled repeatedly over the course of 4 months. At the 3 repeatedly sampled sites, leaf litter bags were also deployed to assess differences in decomposition rates and associated macroinvertebrate community structures as determined by metabarcoding. The results of this study will have a large impact on how agencies choose to conduct routine stream monitoring as continued advances are made in the field of eDNA. Additionally, the expanded biodiversity information provided by eDNA will allow land managers to make more informed decisions about the efficacy of remediation strategies deployed in the sampled streams.

06.01.09 Mine Sediment Ponds Affect Selenium Speciation and Bioaccumulation

A.M. deBruyn, Golder Associates, Inc.; S.N. Luoma, University of California, Davis / John Muir Institute of the Environment; C. Good, Teck Coal Limited; M.C. Arnold, Duke University

Selenium occurs in natural surface waters as a variety of inorganic and organic chemical species. The oxyanions selenate and selenite typically predominate. Organoselenide species, although hypothesized to be more bioavailable than the oxyanions, have rarely been identified or quantified in natural waters and little is known about their fate or bioaccumulative potential. We analyzed spatial patterns of bioaccumulation in relation to aqueous selenium speciation at more than 100 sites in southeast British Columbia, Canada. We used sites with no detectable organoselenium (< 0.01 μg/L) to derive bioaccumulation models for selenate and selenite, then applied those models to the remaining sites to infer the bioavailability of the detectable organoselenides. Our analysis indicated that the methylated species dimethylselenoxide and methylseleninic acid are substantially more bioavailable than selenate or selenite. These organoselenides were associated primarily with mine sediment ponds, presumably as degradation products of selenium metabolism by algae and/or bacteria. Organoselenides exported from the ponds appear to be responsible for enhanced bioaccumulation in biota in downstream reaches. Our findings indicate that managing biological productivity in mine sediment ponds could help manage selenium risk in the receiving environment.

06.02 Portland Harbor: Site Characterization and Risk Assessment to Lay Groundwork for Remedial Design

06.02.02 Successful Willamette River Sediment Remedy Construction at the Former Portland Gas Manufacturing Site, Portland, Oregon

T. Thornburg, Anchor QEA, LLC; B. Wyatt, NW Natural; S. Greenfield, Oregon Department of Environmental Quality; K. Skellenger, Anchor QEA, LLC; M. Crystal, Sevenson Environmental Services, Inc.

Portland Gas Manufacturing (PGM) is a sediment cleanup site at river mile 12.2 on the west side of the Willamette River, 0.5 mile upstream from the Portland Harbor Superfund Site. PGM was a manufactured gas plant that operated between 1860 and 1913. Upland groundwater and river sediments and porewater adjacent to the former plant were impacted by petroleum hydrocarbons, polycyclic aromatic hydrocarbons, and other contaminants. From June to October, 2020, the PGM remedial action was completed under oversight by the Oregon Department of Environmental Quality in accordance with the 2017 PGM Record of Decision and the 2020 PGM Remedial Design. Challenges and successes of the PGM remedial action may inform future downstream cleanup actions in the Portland Harbor Superfund Site. Long-term ecological and human health risks were addressed by the remedial action, which included hot spot and navigational dredging, debris removal, reactive caps with granular activated carbon (GAC), armor rock, sand covers, monitored natural recovery, long-term monitoring, and institutional controls. Construction verification showed that the constructed remedy met or exceeded all design criteria. Careful GAC saturation and submerged placement methods maximized GAC delivery and in-situ GAC content in the sediment cap. Dredge design elevations were achieved, despite abundant debris, and measured cap thicknesses generally met or exceeded minimum required specifications. A dedicated multibeam bathymetric surveyor and custom-coded, GIS-based processing software helped visualize and expedite dredging and capping verification and acceptance. Short-term environmental risks were controlled by water quality and air quality monitoring programs. Turbidity benchmarks and chemical water quality criteria were met during the entire project, largely attributed to the use of a mobile, moon pool curtain system during dredging. There were no air quality concerns in either the work area or Waterfront Park. However, several other unforeseen short-term risks were encountered and managed during construction. These included the COVID-19 pandemic, unprecedented wildfire activity and associated hazardous air quality, and the discovery and management of World War II munitions in dredged sediment. Frequent and candid communications with DEQ and the agility allowed by a design-build arrangement were key to successful, expedited management of these unforeseen short-term risks.

06.02.03 Probabilistic Risk Assessment of Sustainable Fish Consumption at Portland Harbor Superfund Site

B. Ruffle, AECOM / Environment; E.B. Morrison, Arcadis U.S., Inc. / Environment; D. Pfeiffer, ARCADIS US, Inc.; G. Kirkwood, AECOM / Remediation Consulting and Engineering; P.D. Anderson, ARCADIS US, Inc. / Environment

Previously published work (Pfeiffer and Anderson 2020) described a methodology for estimating the amount of edible fish that can be harvested sustainably from a contaminated sediment site by integrating the total fish productivity of an impacted water body, the fraction of fish that can be harvested sustainably, the portion of the sustainable harvest that is edible fish tissue, and the size of the population of consumers. A probabilistic approach was applied to this sustainable fish consumption rate methodology to generate distributions of fish consumption rate and human health risk from polychlorinated biphenyls (PCBs) for the Portland Harbor Superfund Site (PHSS) located on the lower Willamette River in Oregon. The analysis found that Portland Harbor can only support a few subsistence consumers, when the available sustainable fish for consumption is distributed among the population of consumers. At the 95th percentile, which is often considered a reasonable maximum exposure estimate, the sustainable usual fish consumption rate (UFCR) at PHSS is 14 grams/person/day. This upper-bound rate is ten-fold lower than the point estimate fish consumption rate of 142 grams/person/day used for the subsistence angler that is the foundation of the sediment remedy selected for PHSS. A sensitivity analysis of key assumptions supports the approach and its application to other sites. A similar analysis performed for a small freshwater pond (Koppers Pond Superfund Site in Horseheads, NY) also found that the vast majority of consumers have potential risks within or below EPA risk targets used in remedial decision-making. The combination of sustainable fish consumption rates and probabilistic methods results in estimates of potential cancer risk and noncancer hazard that are substantially lower than deterministic estimates calculated using standard risk assessment methods. When sustainable harvest is taken into account, the resulting fish consumption rates are lower than typical default fish consumption rates that assume unlimited quantities of available fish. An advantage of estimating sustainable fish production is the ability to develop estimates of the total number of additional cancers that might occur as a result of exposure to constituents at a contaminated sediment site. By multiplying the distribution of potential excess lifetime cancer risks by the size of the potential consumer population, a maximum of about 1 additional cancer in 70 years is estimated at PHSS.

06.02.04 Evaluation of Buried Contamination Transport Potential to Delineate Active Remediation Areas at Port of Portland, Terminal 4

D. Reidy, Anchor QEA, LLC / Environmental Sciences; A. Shellenberger, T. Thornburg, K. Russell, Anchor QEA, LLC

Remedial options at contaminated sediment sites fall into one of three general categories: no action, natural recovery, or active remediation. In depositional areas where surface sediment concentrations have declined over time, leaving higher concentrations at depth, chemical stability of this buried contamination is an important consideration in the selection and design of in-place remedial technologies, and specifically monitored natural recovery (MNR). At Port of Portland Terminal 4 (T4), buried contamination was rigorously evaluated through modeling studies to identify whether the sediments were chemically stable, which would verify that the contamination would remain buried, allowing natural recovery processes to continue. Contaminant fate and transport modeling was conducted for the purpose of quantifying the migration potential of buried contamination via porewater to surface sediment over a 100-year time frame. Modeling was conducted for polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dioxins/furans, and DDx. A simple transport analysis was conducted as an initial screening step to estimate the distance over which the chemicals of concern at T4 would be expected to migrate via advection alone, factoring in retardation associated with sorption processes. Chemicals predicted to migrate more than a nominal distance were further evaluated in a second, more detailed step using a numerical transport model that accounts for additional transport processes and site-specific core profiles. Five core profiles representing a range of cases, including relative worst-case conditions (i.e., locations with the highest concentrations or elevated concentrations closest to the surface), were selected for the modeling study. The modeling predicted that PCBs, DDx, dioxins/furans, and in most cases PAHs are chemically stable when buried below a 2-foot depth in the sediment column. These chemicals were predicted to migrate at most a few inches in 100 years. Some of the lighter-weight PAHs were predicted to have higher mobilities and will need to be further evaluated on a location-specific basis. This analysis was critical for delineating active remediation areas from areas that would be amenable to MNR, where buried contamination is not predicted to recontaminate surface sediments.

06.02.05 Subsurface Visualization Using Geophysical Survey Methods

K. Kirkland, L. Uselman, Integral Consulting, Inc.; D. Silva, EVRAZ

The River Mile 2 East (RM2E) Project Area, located at the downstream end of the Portland Harbor Superfund Site, is on the inside bend of a broadening curve within the Willamette River and downstream of the Multnomah Channel diversion. The RM2E Project Area has a distinct geomorphic, hydrologic, and hydrodynamic setting that results in high rates of sediment deposition and a sediment bed composed of fine-grained materials. Remedial design for sediments in the RM2E Project Area requires a detailed understanding of subsurface lithology, sediment structures, and debris or obstacle conditions. Core samples are typically used to obtain subsurface data and the RM2E Project Area has high pre-design investigation core sampling density arranged in a 150-foot grid. Discrete point data can be augmented by modeling to evaluate the vertical and lateral extents of chemicals of concern and characterize lithologically complex areas.A geophysical resistivity survey is a highly effective and efficient alternative that can produce a multidimensional model of the subsurface, including sediment layering, lithology, and location of debris or obstacles. In a geophysical resistivity survey, each geologic material has a unique resistivity value. Sediment types and thicknesses are determined by injecting an electrical current into the subsurface and measuring voltage gradients via electrodes towed on a submarine cable. Following the survey, geophysical data are correlated with existing lithological data from boring logs to develop multidimensional models that depict subsurface lithology and features, while also helping to address data gaps related to subsurface conditions. Geophysical data collected in the RM2E Project Area in June 2021 will be used to develop a detailed multidimensional model of subsurface lithology that informs remedial design in several important aspects. The model will be used to examine the relationship between chemicals of concern, grain size, and lithology; to document obstacles and debris in the RM2E Project Area including buried infrastructure; and potentially to identify subsurface nonaqueous-phase liquid in the sediments. This geophysical model will identify features that could adversely affect potential remedial actions and will support selection of appropriate remedial technologies for the RM2E Project Area.

06.03 Puget Sound: Cleanup Sites: Risk Assessment and Management, Remedial Actions and Restoration

06.03.01 A Journey into Puget Sound—Its Ecological Beauty and the Science Informing Cleanup

C. Asher, B. Brooks, Washington State Department of Ecology / Toxics Cleanup Program; J. Evered, Washington State Department of Ecology

Puget Sound, part of the Salish Sea, includes 2,500 miles of marine shoreline and hosts a unique ecosystem facing increasing impacts from urbanization. Its distinctive series of fjords, bathymetry, and shallow sills result in restricted rates of water flushing and continual cycling of persistent, bioaccumulative contaminants from sediment and water through the food chain of benthic communities, fish, birds, and marine mammals. The Southern Resident Killer Whale (SRKW) community is in decline as a result of contamination and a decreasing Chinook salmon population which is their main food source. Studies show a high body burden of PCBs, DDT, and PBDEs in male SRKWs, substantially higher than Resident Killer Whales in Alaska. In 2018 an SRKW, Tahlequah, carried her newly deceased calf throughout the Salish Sea for 17 days—a reminder of their vulnerability. Forage fish species such as Pacific sand lance, Pacific herring, and surf smelt are essential food sources for higher trophic levels. Their spawning ground habitat includes upper beaches and nearshore and subtidal eelgrass and microalgae beds. These critical habitats are highly impacted by contaminated sediment and upland cleanup sites as well as habitat degradation. Washingtonians, including over 20 federally recognized tribes with Usual and Accustomed Fishing Areas in Puget Sound, eat a high amount of fish and shellfish from Puget Sound and rely on healthy and abundant harvests. Puget Sound is also vulnerable to climate change impacts—particularly sea level rise, increasingly severe storms and flooding, and ocean acidification—which present challenges to remedy resilience. This presentation will introduce the unique ecosystem of Puget Sound, the pressures impacting its environment, and the science informing regulatory policy and cleanup decisions. The focus will be on how the state is implementing the Puget Sound Initiative to protect human health and the environment by cleaning up persistent, bioaccumulative chemicals, emphasizing active cleanup, reducing contaminant loading from stormwater, and making habitat restoration a priority.

06.03.03 Climate Change and Cleanup - Vulnerabilities for Contaminated Sites in Puget Sound

C. Asher, Washington State Department of Ecology / Toxics Cleanup Program

Puget Sound in Washington state is one of the largest estuaries in the United States with over 2500 miles of marine shoreline. Most of the state's population lives near Puget Sound, which has resulted in a majority of contaminated sites located in Puget Sound and along the shoreline. These include sediment, soil, groundwater, and mining sites, failed landfills, and underground storage tanks (i.e., operating gas stations or leaking tanks). Our ability to improve the resilience of cleanup remedies and respond to the impacts associated with climate change is important to: · Protect human health and the environment - which includes plentiful aquatic wildlife and resources,· Ensure cleaned up sites are protective and effective over the long-term, and · Protect the significant financial and resource investment required to conduct cleanup. Ecology conducted a vulnerabilities assessment of the state's contaminated sites to identify the climate change impacts that posed the highest threat. Our approach included:Using best available climate science from the Intergovernmental Panel on Climate Change, NOAA, EPA, and University of Washington Climate Impacts Group,Developing a GIS analytical tool and risk scenarios to determine which climate change impacts posed the highest threat,Identifying the most vulnerable cleanup sites and remedies, andDetermining the scientific and technical criteria necessary to increase resilience at different phases of the cleanup process.This presentation will include the results of our vulnerability assessment and the resulting adaptation strategy we developed to increase the resilience and sustainability of the state's contaminated sites. Due to the vulnerabilities of remedies with contained contamination, a robust and innovative adaptive management approach will be necessary to sustain remedies for the long-term, which will require new and dynamic thinking.

06.03.04 The Port Gamble Mill Site Cleanup - Site History, a Completed Sediment Remedial Action, and Remedy Scope Changes

J. Evered, Washington State Department of Ecology

Timber industries in Washington State have played an important role in the state's economic and industrial development, with mills producing pulp, paper, plywood and other timber products. The Port Gamble Mill, located on Puget Sound, specifically Port Gamble Bay in Washington operated for 142 years from 1853 until its closure in 1995. Areas surrounding the former mill site are largely rural with the Port Gamble S'Klallam Tribal reservation located to the east of the Bay. Tribe members actively use the Bay for shellfish harvesting, fishing and other resources. Contamination at the site is related to the use of the sawmill, the operation of two chip barge loading facilities, sawmill emissions of particulates from burning of wood and wood waste, and several thousand creosote pilings present throughout the Bay used to support Mill structures. In addition to accumulations of wood waste and its breakdown products in subtidal areas surrounding the former Mill site, elevated concentrations of cadmium, carcinogenic polycyclic aromatic hydrocarbons and dioxin/furans in marine sediments presented a threat to human health and the environment, requiring remedial action. The Port Gamble cleanup objectives were to reduce surface sediment toxicity to the benthic environment as a result of wood waste degradation, and reduce cPAHs to background sediment concentrations across a 710-acre subtidal area. This presentation will provide an overview of the historical mill operations at Port Gamble, the completed sediment cleanup remedy and a description of the remedy scope changes that were necessary during cleanup.

06.03.05 Sediment Cleanup Lessons Learned: Port Gamble Bay

C. Patmont, Anchor QEA

Between 2015 and 2017, the in-water construction phase of the Port Gamble Bay Cleanup project was successfully completed, including:Removal of 8,500 decayed piling, nearly all of which were creosote treatedRemoval of 110,000 cubic yards of wood debris and sedimentRemoval of 1.3 acres of overwater and derelict structuresPlacement of 87 acres of clean sand caps and coversImprovement of 0.7 miles of shorelineDetailed monitoring was performed throughout remedy implementation and for 3 years following completion of construction to verify the protectiveness of the remedy, including controlling contaminant sources, reducing surface sediment toxicity and bioaccumulation, and accelerating natural recovery throughout the 710-acre bay. Key monitoring parameters included sediment chemistry, sediment toxicity, shellfish tissue chemistry, and paralytic shellfish poisoning indicators.

This presentation will summarize the results of Port Gamble Bay remedy effectiveness monitoring, focusing on sediment and biological parameters. Overall lessons learned from the cleanup project will also be highlighted, including:Cooperative engagement, project planning, and implementation with local tribesSelection of highly qualified construction contractors and adaptive management with stakeholdersPilot studies of key remedial technologiesPermitting a local limited purpose landfillIntegration of cleanup and habitat restoration

06.03.06 Process Based Nearshore Restoration with the Port Gamble S'klallam Tribe

D. Elefant, Environmental Science Associates / Ecohydrology

The Port Gamble, WA former mill site is a traditional cultural site for the Port Gamble S'Klallam Tribe (PGST). It has a more recent legacy of pollution (dioxins, furans, and others) that persists to this day. Ongoing cleanup efforts are in the design and construction pipeline for the near future and several phases of aquatic cleanup have occured. PGST has engaged engineers and nearshore restoration specialists to advise and plan for PGST's long-term vision for a stable and clean site that provides habitat for species such as forage fish, eelgrass, gooey duck, salmon, and others. Naturally, these types of cuspate sand spits are formed at sediment transport convergence zones. The position, shape, and stability of the tip of the spit is dependent on incident wave conditions, nearshore currents, and the flow of sediment reaching the spit from updrift shorelines. Littoral drift of sediment along the northern shoreline of Port Gamble flows from west to east and from south to north along the southeastern shoreline. This presentation will focus on the challenge of designing a nearshore restoration that is process-based while feasible for contaminant removal and capping in-place. The restoration design seeks a balance between sustainable shoreline habitat features and stable long-term contaminated sediment caps that could be exposed to wave action.

06.03.07 Finding the Sources of PCBs in Upland Groundwater: Is It From Contaminated Sediment?

M. Alam, WA Dept of Ecology

Polychlorinated biphenyls (PCBs) are one of the persistent, bioaccumulative, and toxic contaminants found in many hazardous waste sites. An investigation was launched to find sources of PCBs in upland groundwater for a site in Port Gardner Bay near Everett, Washington. During remedial investigation, PCBs were found in unusually high levels (more than 1000 times the applicable surface water quality standards) in the groundwater seeps discharging during low tides. Geoprobe borings, test pits, and monitoring wells were constructed to locate the sources in the upland area. Results from this investigation revealed a PCB plume in upland groundwater but with no apparent sources in the vedose zone soil. PCBs were analyzed with EPA method 1668 (PCB congeners analysis with high resolution mass spectrometry) to achieve very low detection level and source tracing. The intertidal area near the seeps were also contaminated with PCBs. Another investigation was launched to find out whether upland groundwater is acting as a source to the nearby sediment. Slotted stainless steel sampling tubes loaded with solid-phase microextraction (SPME) fiber were deployed in sediment porewater near seep discharge locations as well as in upland monitoring wells to find time-averaged concentrations of freely dissolved PCBs. Results from the in-situ SPME sampling revealed freely dissolved PCBs in the seep location porewater are 10 to 30 times higher than upgradient groundwater in monitoring wells. These results suggested contamination of upland groundwater could be from historically contaminated sediment due to tidal action and upland groundwater may not be a source of PCBs to intertidal sediment. As such, in the cleanup feasibility study, no active groundwater remedy is suggested. We expect that the marine sediment remedy, which will remove top 2 ft. of contaminated surface sediment from the area, will reduce the levels of PCBs in the upland groundwater. Upland groundwater and sediment will be part of the long term monitoring to verify the conceptual site model and verify sediment remedy effectiveness.

06.03.08 Utilizing Interim Actions to Expedite Cleanup Process at the Bay Wood Products Cleanup Site in the Snohomish River Estuary in Everett, WA

S. Meng, Washington Department of Ecology

Site cleanups of contamination from historical operations under state or federal programs often take many years to complete. Delineating the contamination is time-consuming because the contaminants may have spread over the site. On the contrary, a contaminant introduced to a site recently, by a known source, is normally easier to delineate, unless it is highly mobile. Therefore, the cleanup decision for the newly introduced contaminant sometimes can be made quicker, potentially allowing a faster action. An interim action is an early action that could take place before the decision is made for the whole site allowing a relatively quick response when it is necessary, minimizing the risk to the humans and the environment. Under the oversight of the Washington Department of Ecology, two interim actions were conducted at the Bay Wood Products Cleanup Site – a contaminated site located where the Snohomish River meets the Port Gardner Bay. Polychlorinated biphenyls, polycyclic aromatic hydrocarbons (PAHs), metals, and dioxins/furans associated with historical site operations and a stormwater outfall were detected at levels exceeding their respective state cleanup standards in soil and sediment. Unexpected sources of PAHs and dioxins/furans, which both appeared to impact site for a short time period, were discovered while the cleanup decision was still under discussion. This presentation covers the discovery and delineation of the two unexpected sources, and subsequent two interim actions designed to remove the soil that was impacted.

06.03.09 Shoreline Restoration at the Bay Wood Products Cleanup Site in the Snohomish River Estuary in Everett, WA

S. Edwards, Washington Department of Ecology / Toxics Cleanup Program

Contaminated sites present unique challenges for habitat restoration. Restoration practitioners must be aware of the nature and extent of contamination and of criteria and remediation plans developed to protect human and ecological health. This knowledge allows them to incorporate restoration plans that are compatible with the cleanup design and minimize risks from pollutants. This presentation focuses on restoration of shoreline habitat at the Bay Wood Products Cleanup Site – a contaminated waterfront property along the Snohomish River estuary in Everett, WA. Decades of industrial use degraded the Site's shoreline which is adjacent to a critical, high-use juvenile salmonid mudflat habitat designated as "Aquatic Conservancy". For over half a century, the Site was used for sawmilling, timber rafting, and log storage operations. Those activities created accumulations of wood waste and other anthropogenic debris in the shoreline soil and tidal mudflats. Historic industrial operations at the Site and adjacent properties released pollutants including PCBs, PAHs, arsenic, and dioxins/furans to soil and sediment. In 2020 and 2021, the Port of Everett conducted a Model Toxics Control Act Interim Action to clean up a portion of the Site and restore riparian habitat for fish, birds, and other wildlife. This presentation covers the Interim Action's restoration goals and design, describes how the team addressed site-specific challenges, and highlights the construction outcomes. Executed with oversight from the Department of Ecology, this project shows how cleanup, restoration, and redevelopment can occur at one location.

06.03.10 Remediation and Restoration as Drivers of an Environmental Cleanup at a Former Plywood Mill in Anacortes, WA

J. Shannon, J. Blanchette, M. Ehlebracht, Hart Crowser, a division of Haley & Aldrich; H. Park, Washington Department of Ecology; A.L. Fernandez, Washington State Department of Ecology; S. Edwards, Washington Department of Ecology / Toxics Cleanup Program; J. Bingham, A. Kaparos, Hart Crowser, a division of Haley & Aldrich

The Custom Plywood Remediation project, a Puget Sound Initiative site, provides a rare opportunity to meaningfully restore a historical industrial waterfront as part of broader site-wide cleanup action. Implementation was phased based on cleanup actions and available funding, ultimately resulting in subtidal, intertidal, and nearshore excavation and restoration in 2013. As part of these cleanup activities, Hart Crowser, a division of Haley & Aldrich, designed a beach with Ecology that protects the area from capped residual contamination remaining in the upland portion of the site, and also restored historical ecological function to the nearshore. The beach design promoted forage fish spawning and use by outmigrating salmonids, and restored emergent nearshore/wetland plants as part of a pocket estuary. Performance surveys in 2014 and 2015 quantitatively examined salmonid use, epibenthic zooplankton productivity, forage fish spawning occurrence/success, and wetland plant recruitment. The monitoring results show increased use, activity, and productivity along the beach and within the estuary from year to year compared to an adjacent unrestored shoreline. Not only did we remove contamination that was a potential human health risk, but we also improved physical and ecological processes at the beach. Our remediation design has restored ecological function to the Fidalgo Bay system, after over a hundred years of industrial waterfront damage.

06.03.11 Custom Plywood Restoration-An Indigenous Perspective from the Samish Indian Nation

T. Woodard, Samish Indian Nation / Natural Resoruces

Samish People have been stewards of their Traditional Territory since time immemorial. The Salish Sea has provided sustenance and cultural use materials since that time and continues to do so today. As the City of Anacortes was settled and developed, the new industries provided jobs and prosperity to all of the community both Indigenous and non indigenous alike. But there was a cost. Legacy pollution has had an impact and continues to impact species culturally significant to the Samish People. The Samish Department of Natural Resources' mission statement is to "Protect, Enhance , and Preserve all Culturally Significant Natural Resources and their Habitats for Current and Future Generations". Samish Natural Resources has been involved with the Custom Plywood Cleanup since its beginning, providing local meteorological and first hand knowledge of the area during design, and currently provides water quality and fish population study support for the project. This talk will integrate an Indigenous perspective to the clean up providing context and rationale for Tribal Support of the project.

06.03.14 Activated Carbon-Amended Enhanced Natural Recovery (ENR): Results of a Pilot Study in the Lower Duwamish Waterway

J.M. Conder, Geosyntec Consultants; V.S. Magar, Ramboll / Ecology Sediment Management; G. Heavner, Floyd|Snider; L.C. Nelis, Ramboll; C. Whitmus, Wood; E.C. Revelas, Intergral Consulting, Inc.; A.P. Wang, Geosyntec Consultants, Inc. / Civil & Environmental Engineering; D. Williston, J. Stern, King County Department of Natural Resources and Parks / Department of Natural Resources and Parks; L. Erickson, Boeing; J. Flaherty, The Boeing Company; D. Schuchardt, P. Rude, A. Crowley, City of Seattle; J. Florer, Port of Seattle

The use of Activated Carbon (AC) to augment Enhanced Natural Recovery (ENR) is an increasingly recognized remedy to reduce the bioavailability of hydrophobic, bioaccumulative compounds. A pilot study conducted from 2017 to 2021 to compare the effectiveness of ENR and ENR amended with 3-4% (by weight) granular AC (ENR+AC) in the Lower Duwamish Waterway, Seattle, WA. In 2017, parallel ENR and ENR+AC plots were placed in three, one-acre plots: one in a deeper navigation channel, one in a berthing area subject to scour, and one in an intertidal location subject to waves and wakes. Monitoring events included pre-construction baseline sampling (2016), post-construction monitoring (2017), and three years of monitoring (2018, 2019, and 2020). Evaluations focused on ENR and AC stability, PCBs in bulk sediment, PCBs in porewater (C_{free}, freely dissolved) using passive samplers, biological conditions using Sediment Profile Imagery (SPI), a laboratory bioaccumulation study, and a benthic community survey to evaluate the potential impact of AC on benthic animals. The key study finding was that differences in PCB C_{free} were insubstantial or not statistically significant when comparing ENR and ENR+AC for all three treatment areas. ENR reduced PCB bioavailability so well (e.g., 90% or more from baseline) that no major improvement could be detected from adding AC. Additionally, ENR and ENR+AC plots were reasonably stable under the range of environments tested. The AC content of the ENR+AC layer remained present at percentage levels in all three ENR+AC subplots, with decreases over time due to dilution of the layers with natural sediment deposition. PCBs in clams and polychaetes exposed in a laboratory bioaccumulation test were not statistically different between ENR and ENR+AC, confirming no significant PCB-bioavailability differences between ENR and ENR+AC. Results did not indicate adverse effects due to the added AC, as there were no differences between ENR and ENR+AC between: 1) survival or growth of bioaccumulation test clams and polychaetes; 2) multiple benthic macroinvertebrate community survey metrics, including abundance of Annelids, which are considered uniquely sensitive to AC; and 3) benthic recolonization and development of Stage 3 infaunal communities.

06.03.15 Activated Carbon-Amended Enhanced Natural Recovery (ENR): Biological Lines of Evidence from a Pilot Study in the Lower Duwamish Waterway

J.M. Conder, Geosyntec Consultants; E.C. Revelas, Intergral Consulting, Inc.; A.P. Wang, Geosyntec Consultants, Inc. / Civil & Environmental Engineering; V.S. Magar, Ramboll / Ecology Sediment Management; G. Heavner, Floyd|Snider; L.C. Nelis, Ramboll; C. Whitmus, Wood; D. Williston, J. Stern, King County Department of Natural Resources and Parks / Department of Natural Resources and Parks; L. Erickson, Boeing; J. Flaherty, The Boeing Company; D. Schuchardt, Seattle Public Utilities; P. Rude, A. Crowley, City of Seattle; J. Florer, Port of Seattle

The use of Activated Carbon (AC) to augment Enhanced Natural Recovery (ENR) is an increasingly recognized remedy to reduce the bioavailability of hydrophobic, bioaccumulative compounds. As part of a pilot study conducted from 2017 to 2021, we evaluated the potential for AC-associated effects to benthic invertebrates using a comparison of multiple biological lines of evidence at 3 subplots amended with ENR and 3 subplots amended with ENR containing 3-4% (by weight) granular AC (ENR+AC) in the Lower Duwamish Waterway (Seattle, WA, USA). Results indicated that AC did not adversely affect benthic invertebrates. The three annual Sediment Profile Imaging/Plan View surveys (2018-2020) indicated no consistent differences in benthic community colonization between ENR and ENR+AC subplots. The percentages of Stage 3 benthic communities, characterized by deep dwelling, head-down deposit feeding benthic invertebrates, in 2020 in the ENR subplots were neither consistently higher nor lower than in the ENR+AC subplots for the intertidal (25% vs 25%), scour (100% vs 67%), or subtidal (25% vs 67%) plots; differences observed were attributed primarily to differences in natural sedimentation or scour in each respective subplot. These results were reinforced by the laboratory test results. In 2020, a 28-day laboratory bioaccumulation study in which polychaete worms (*Nephtys caecoides*) and bivalve clams (*Mya arenaria*) were exposed to core samples obtained from one adjacent pair of ENR and ENR+AC subplots. Mean wet weight organism masses measured at the end of the exposures were not significantly different ($p > 0.05$) between ENR and ENR+AC for either clams (7.0 vs. 7.3 g/individual) or polychaetes (0.33 vs. 0.31 g/individual). Mean survival was not significantly different ($p > 0.05$) between ENR and ENR+AC for either clams (99% vs. 93%) or polychaetes (94% vs. 92%). Also in 2020, a benthic macroinvertebrate survey evaluated multiple metrics (abundance, Annelid abundance, richness, diversity, evenness, dominance, composition of major taxa) at the 6 subplots. Among 18 statistical comparisons of the metrics, only four indicated a significant difference ($p < 0.05$) between ENR and ENR+AC; these differences could be explained by disparate physical conditions and silt deposition between the ENR and ENR+AC subplots.

06.03.16 Utility of Method 8082 Data for PCB Forensics at a Site with Limited Congener Data: A Case Study from the Lower Duwamish Waterway, Seattle, WA

G.W. Johnson, University of Utah / Energy & Geoscience Institute

The Lower Duwamish Waterway (LDW) is an industrialized river in Seattle, WA that flows north into Elliott Bay and Puget Sound. The lower five miles of the Duwamish is a Superfund Site, with polychlorinated biphenyls (PCBs) as one of the primary chemicals of concern and remedial drivers. While RI/FS and Pre-Design databases include PCB data for thousands of sediment samples, the vast majority of those were analyzed by USEPA Method 8082 (i.e., "Aroclor" data). Method 8082 data are useful in identifying degree and extent of PCB contamination, but has limitations in environmental forensics, especially as compared to full congener data (USEPA Method 1668A). In this study we apply a quantitative mixing model to the limited full congener data sets from the LDW and compare the observed congener profiles to Aroclors reported by Method 8082 in the same samples. With the exception of sediment from the head of one of the waterways slips, PCB fingerprints in LDW sediments reflect mixtures of typical, unaltered commercial PCB products. Comparison to Method 8082 results in the same samples shows that "Aroclor" analysis was effective in identifying major contributing sources in sediment, but often missed more nuanced fingerprints apparent in the full congener data (e.g., source fingerprints present in small percentages, and microbial dechlorination). While full congener data are decidedly preferred for source apportionment and forensics, this analysis makes clear that in absence of full congener data, Method 8082 can provide useful insight into PCB sources in environmental forensics investigations.

06.04 Engineering, Remediation and Restoration

06.04.01 A Reliable Dataset from a PFAS Sediment Investigation Near a Former (Confidential) Manufacturing Site

M. Hayes, R. Beach, K. McDonald, L. Nelson, M. Westra, GZA GeoEnvironmental, Inc.

Per- and polyfluoroalkyl substances (PFAS) in the environment have become the subject of substantial interest over the past decade. Concerns about the environmental and health impacts of this group of compounds have emerged due to their varying potential toxicities and persistence in the environment. There have been many challenges preventing investigations from developing a good understanding of PFAS distributions in the sediment environment, including the ubiquitous presence of PFAS, cross-contamination during sampling, suitable analytical methods, appropriate field and laboratory QA/QC, and the usual challenges of sediment investigations in complex and heterogeneous substrates. The majority of recent presentations and training programs on PFAS in sediments that do not involve toxicity studies have focused on analytical methodology, hypothetical partitioning considerations, and theoretical discussions of where PFAS should reside in sediments instead of providing comprehensive site evaluations. This presentation, however, will describe the reliable and substantial dataset of sediment PFAS that was developed as part of a broader investigation at a legacy site for a confidential client. Over 99 high-quality sediment samples were collected during the investigation from a river and a tributary creek using methods developed based on our experience from PFAS groundwater and soil investigations and modified based on our experience with sediment investigations. The samples were primarily generated from six-foot cores collected by vibracoring methods along 10 transects, with 3 locations per transect, and up to 4 sample intervals per core. The sediment cores were generally retrieved intact and consisted of primarily sandy silt with larger amounts of gravel and organic matter at various locations and sediment depths. In addition to potential contaminants of concern (COCs) evaluated as part of the broader site investigation, twenty-three PFAS compounds were analyzed in each sediment sample with detection limits as low as 0.43 ug/kg and a maximum concentration of 170 ug/kg perfluorooctane sulfonic acid (PFOS) using the U.S. DOD QSM isotope dilution method. The spatial and vertical distributions of total PFAS and select PFAS compounds in sediments will be presented that illustrate the narrow distribution of PFAS near the shoreline.

06.04.02 Abiotic Reduction and Sorption of the Insensitive Munitions Constituents by Wood-Derived Biochars

D. Xin, J. Girón, University of Delaware / Department of Civil and Environmental Engineering; M.E. Fuller, Aptim Federal Services, Inc.; P.C. Chiu, University of Delaware / Civil and Environmental Engineering

Department of Defense (DoD) has developed insensitive munitions constituents (MCs) to replace legacy MCs. 3-Nitro-1,2,4-triazol-5-one (NTO), nitroguanidine (NQ), and 2,4-dinitroanisole (DNAN) are the three major constituents in IMX-101 formulation. NTO and DNAN are also the main constituents of IMX-104 formulation. Although insensitive MCs are safer and less prone to accidental detonation than legacy munitions compounds, they are more water-soluble and hence highly leachable in the environment. These insensitive MCs can be mobilized during storm events and may exist at elevated concentrations in surface runoff from active ranges. There is a need to develop effective and inexpensive materials that can be used in stormwater treatment systems to remove and degrade insensitive MCs in DoD testing sites and training ranges. Biochar is a class of engineered black carbon produced through pyrolysis of surplus biomass. Biochar can be an effective sorbent for organic solutes and an electron donor to support abiotic and microbial redox reactions, due to its significant specific surface area and electron storage capacity (ESC), respectively. In this study, we investigated the efficacy of two wood-derived biochars (Rogue and Soil Reef biochars) to remove or chemically reduce insensitive MCs (NTO, NQ, and DNAN). We conducted batch experiments in buffered solutions at pH 6–10 and in artificial stormwater runoff at pH 6. Parallel experiments were performed using dithionite-reduced (ESC-filled) biochar as an electron donor for the abiotic reduction of MCs and air-oxidized (ESC-depleted) biochar for non-reactive sorption control. Results showed that, while inert to NQ, reduced biochars were able to abiotically reduce NTO and DNAN through its ESC. NTO removed by oxidized biochar was minimal, because NTO is anionic at circumneutral pH and was hence negligibly sorbed. In contrast, when exposed to reduced biochar, NTO was removed to a significant extent and 3-amino-1,2,4-triazol-5-one (ATO) was formed concomitantly, confirming that NTO was chemically reduced to ATO. The amount of ATO formed by reduced Soil Reef biochar was approximately 90 μmol/g and was constant in the pH range of 6–10 in the buffered system. In artificial stormwater, both Soil Reef and Rogue biochars produced 95 μmol/g ATO through reduction. For DNAN, up to 500 μmol/g of DNAN was removed by oxidized Rogue biochar. When exposed to the reduced biochar, an additional 100 μmol/g of DNAN was removed beyond sorption, suggesting the reduced biochar may be reactive towards DNAN. DNAN reduction was also confirmed by detecting a total of 70 μmol/g of 2-amino-4-nitroanisole and a trace amount of 4-amino-2-nitroanisole (< 2 μmol/g) from aqueous and solid phases. This shows that DNAN can be removed by biochar through concurrent sorption and reduction, but primarily through sorption. This study demonstrates the potential utility of biochar to remove insensitive MCs from stormwater and suggests biochar-based remediation strategies may be developed for DoD sites.

06.04.03 Aquatic Toxicity of Hydroquinone and Catechol Following Metal Oxide Treatment to Ceriodaphnia dubia and Pimephales promelas

M. Abugazleh, Arkansas State University / Chemistry and Physics

Metal oxides comprise a large group of chemicals used in water treatment to adsorb organic pollutants. The effects of titanium dioxide (TiO2) and iron (III) oxide (Fe2O3) on reducing the chronic toxicity of (phenolic) C6H6(OH)2 isomers, namely hydroquinone (HQ) and catechol (CAT) to Ceriodaphnia dubia and Pimephales promelas were investigated.

The toxic endpoints following metal oxide treatment were compared to endpoints of untreated CAT and HQ. Chronic toxicity testing to HQ resulted in greater toxicity than CAT for both test organisms; the median lethal concentrations (LC50) for HQ were between 0.039 to 0.075 mg.L-1 for P. promelas and C. dubia, respectively, while LC50 for CAT were between 13.67 to 3.13 mg.L-1, respectively. Despite both treated solutions displaying significantly lower toxic endpoints than those in the untreated solutions, Fe2O3 had a better potential to reduce the toxicities of CAT and HQ better than TiO2.

06.04.04 Assessment of Transformation and Mitigation of PFAS in Contaminated Brine With nNi0Fe0-Activated Carbon Nanocomposites

M. Modiri Gharehveran, Y.J. Choi, L.S. Lee, Purdue University / Department of Agronomy

Recently, groundwater contamination by poly- and perfluoroalkyl substances (PFAS) has become a significant concern with aqueous fire-fighting foams (AFFFs) as one of the major sources of PFAS release into groundwater. Given the very persistent nature of PFAS in the environment and in the human body, as well as adverse health effects, it is crucially important to develop effective and appropriate remediation technologies for PFAS removal from groundwater. Various methods have been investigated for PFAS removal such as sorption techniques, nanofiltration membrane, and activated persulfate. However, most of these technologies have some drawbacks such as high costs, low efficiency, secondary pollutants, and not being amenable for in-situ application. Previously, we demonstrated the ability of bimetal nanoscale zero valent iron particles (nNi^0Fe^0) synthesized onto activated carbon (AC) in transforming a mixture of perfluoroalkyl acids (PFAAs) in deionized water (DI) in both batch reactors and steady-state flow column studies at 50 and 60 °C. Fluorine mole balance achieved in the column studies approached 73% with fluoride accounting for ~23% of the PFAAs transformed. Here, we did batch and column studies with a PFAS-contaminated brine from an AFFF impacted site. The highest PFAS concentrations identified included a suite of perfluoroalkyl sulfonates (PFSAs), perfluoroalkyl carboxylates (PFCAs) and 6:2 FTS. Additional experiments included batch studies of PFAS-containing DI water solutions compared to electrolyte compositions and at PFAAs/6:2 FTS ratio similar to the brine. Preliminary results showed lower % transformation of perfluoroalkyl sulfonates (PFSAs, C4, 6, and 8) in the brine compared to a DI water. For example, ~70% perfluorooctane sulfonate (PFOS) removal from DI water compared to only ~ 30% from the brine. In both cases, generation of perfluoroalkyl carboxylates were observed; however, the ultra-short chain C2 and C3 PFAAs were only generated in the brine. Batch and column results will be summarized and compared for transformation in brine versus PFAS mixtures in DI water including, % transformation, fluoride and organic product generation, and relative changes in PFAS precursors.

06.04.05 Effectiveness of Fungal Consortium Assisted Mycoremediation Process in Liquid Culture and Soil System Contaminated with Deltamethrin and 3-Phenoxybenzaldehyde

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The widespread use of type-II pyrethroids in agricultural and residential practices has raised serious public health and environmental concerns. The study was conducted with the aim to mycoremediate pyrethroids-contaminated soil using fungal-consortium. Concurrently, the degradation potential of fungi was assessed in 4 regimes (T1-added carbon-medium; T2-carbon-free medium; T3-sterile soil; T4-non-sterile soil; and T5-sterile soil without fungi) supplemented with deltamethrin (DMT) and 3-phenoxybenzaldehyde (3-PBA). Complete removal was achieved for DMT and 3-PBA at 200 mgL^{-1} in liquid medium (T1 and T2) within 15 d. Various parameters were optimized using the central-composite design matrix of Response Surface Methodology; following which quadratic-model was successfully developed. Chromatographic studies were performed to quantitate pesticide residues and metabolite identification. DMT/3-PBA (100 $mgKg^{-1}$) dissipation rates were considerably higher in treated soil system (T3) reaching 84.16 %/ 77% compared to 24.55%/19.03% in T4 and 3.25%/1.97% in T5 within 15 d, respectively. To conclude, this study demonstrates the possible implication of fungal-consortium as an effective remediation strategy for the reclamation of pyrethroid-contaminated environments.

06.04.06 Effects of Biochar-Based Vineyard Practices on Glyphosate Adsorption, Mobility and Fate

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Consumer awareness is rising regarding the presence of pesticide residues in wine – particularly glyphosate. To explore the effects of biochar-based soil amendments on glyphosate mobility and fate, vineyard trials were established in the fall of 2018. Two conventionally-managed Oregon sites were chosen with distinct soil types and climates, but planted with the same plant material. Three treatments were applied under vines at each location after harvest: no biochar; 18 tons ha^{-1} biochar (B18); 35 tons ha^{-1} biochar (B35). Our main hypothesis was that the application of biochar to vineyard soil would reduce the amount of glyphosate transported into grapes, and ultimately into wine. Additionally, the potential translocation of glyphosate from the soil solution through the roots was investigated adding ^{14}C labeled glyphosate to plastic pots containing vine plants. After three days, leaves from potted vines were sampled, digested, and analyzed by liquid scintillation counting. Glyphosate sorption was assessed on the biochar and vineyard soils using the batch equilibration method. Sorption isotherms were generated for the biochar-soil mixtures. Fixed bed column studies were conducted utilizing repacked columns and ^{14}C-glyphosate and analyzed for ^{14}C by liquid scintillation counting. We used the Eurofins Abraxis ELISA assay to quantify glyphosate in the wine. The concentration of glyphosate in leaves from plants treated with 25 mL of 1 ppm ^{14}C glyphosate labeled solution was approximately 10 ng g^{-1}, which confirms the translocation of glyphosate from roots to leaves and explains residual amounts of glyphosate in wines produced from grapes of both locations. The sorption coefficient to the biochar was 84.22 ± 2.86 mL g^{-1}, with a 25.04 ± 11.50 % of sorption. No significant differences were found among the treatments at either location. Sorption of glyphosate to Woodhall soils was approximately 20.8% higher than to Cedars soils, and biochar did not affect the adsorption coefficients at either the single (B18) or at the double application dose (B35) when compared with the unamended soil. The model Pearson correlation (R^2) ranged from 0.873 to 0.988, which indicates good model agreement with the collected leaching concentration data. Glyphosate mobility and, ultimately, the residual amount of glyphosate in wine, was not significantly affected by the addition of biochar.

06.04.07 Evaluating the Ability of Activated Carbon to Reduce DDX Bioavailability Downstream from a Superfund Site

A.D. Harwood, Alma College / Environmental Studies/Biology; S.A. Nutile, Penn State Behrend / Biology; T. Hutchinson, University of Plymouth; F.S. Dillon, Jacobs Engineering Group, Inc. / Global Environmental Services; T. Alcamo, USEPA Region 5

The ability of carbon amendments to reduce the bioavailability of hydrophobic organic contaminants in sediments and soils has been well documented in the literature. However, fewer studies have examined the ability of field applications of carbon sequestration to reduce bioavailability as a remediation strategy in floodplain soils. In a pilot study, a 50 yard by 100 yard area of the floodplain downstream from the Velsicol Chemical Company superfund site (subdivided into 50 grids) was selected based on previous remedial investigations in the area which identified DDT and its metabolites as the primary contaminant of concern. Prior to carbon application, 10 of the grids were randomly selected and surface soil concentrations were analyzed for DDT and its metabolites and organic carbon. To evaluate changes in bioavailability SPMEs were utilized in the laboratory and earthworms were collected from the top 4 inches of soil for tissue analysis. Activated carbon was then added to the test plot at a rate of approximately 2% by weight relative to the top inch of soil. Soil and earthworms were collected at three and nine months post carbon application. In the majority of plots, both the tissue and SPME fiber concentrations were reduced post-carbon application. This indicates that the manual application of activated carbon may be an effective remediation strategy on floodplain soils. Future studies will evaluate the long-term success of this application as well as the ability of SPME for use in long term monitoring of these sites.

06.04.08 Evaluating the Effects of Activated Carbon on Eisenia fetida

R.E. Yates, Alma College / Environmental Studies; M.E. Arkles, Alma College / Department of Chemistry; A.D. Harwood, Alma College / Environmental Studies/Biology

A potential strategy for reducing the bioavailability of organic contaminants in soil to is through the application of granular activated carbon to the soil's surface, due to its ability to sequester organic pollutants. This approach to soil remediation is currently being evaluated in the floodplain downstream of the Velsicol Chemical Superfund site in St. Louis, MI, as the floodplain was contaminated with DDT and its metabolites. However, some St. Louis community members are concerned about the potential toxic effects of activated carbon on the native wildlife. This study evaluated the potential effects of activated carbon on the growth, reproduction, and survival rates of both adult and juvenile *Eisenia fetida*. The activated carbon concentrations used in this study ranged from 0.5% to 10% based on the soil's wet weight. This far exceeded the 2-4% applied in the field. The results of this study indicate that activated carbon has no significant effect on adult or juvenile *E. fetida* growth, reproduction, or survival rates at any of the tested concentrations. While additional studies with other species are required, the application of activated carbon to contaminated soils is unlikely to impact earthworm populations.

06.04.09 Evaluation of Several Adsorbents for Possible In Situ Remediation of PFAS-Contaminated Groundwater

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Per- and polyfluoroalkyl substances (PFAS) are pervasive and recalcitrant contaminants in groundwater. The mitigation of PFAS in groundwater represents a significant liability for the Department of Defense. The US Department of the Navy (DON) is investigating possible technologies to remediate PFAS contamination in situ. This project, funded through the Navy Environmental Sustainability Development to Integration (NESDI) program, aims to evaluate several mature adsorbent technologies for the adsorption of a variety of PFAS (short-chained, long-chained, precursors, etc.) for eventual use as in situ amendments for groundwater PFAS contamination. The second year of this three-year project aims to complete a suite of laboratory studies at environmentally realistic concentrations in groundwater. The effect of pH, background ions, and natural organic matter will be examined using a rapid small-scale column design to understand the effects of these water quality parameters on the performance of granulated activated carbon (GAC) and an anion exchange resin in parallel and in sequence. The results of this study will be presented. Further, a field apparatus designed by NIWC Pacific that aims to allow for onsite sorbent evaluation will be presented with preliminary test results. Results from this project will inform future DON remediation actions for PFAS contaminated groundwater.

06.04.10 Factors Affecting In Situ Immobilization of Per- and Polyfluoroalkyl Substances by Colloidal Activated Carbon

S. Gilak Hakimabadi, A. Taylor, A. Pham, University of Waterloo / Civil and Environmental Engineering

Per-and polyfluoroalkyl substances (PFAS) are a family of over 9000 compounds that are used in numerous applications, including in aqueous film forming foams (AFFF) to extinguish fires at military bases, oil refineries, and firefighting training facilities. The release of large quantities of AFFF at these sites has led to the widespread contamination of groundwater by PFAS, posing a significant risk to drinking water resources. Therefore, there is an urgent need to develop technologies that can prevent the transport of PFAS downstream of the AFFF-impacted sites. The objective of this research is to evaluate the in situ immobilization of PFAS by colloidal activated carbon (CAC) barriers, which are created by injecting a suspension of CAC into the subsurface. As contaminated groundwater passes through CAC, PFAS will be adsorbed by CAC and clean groundwater will flow out of the barrier. However, as with any sorption-based technology, over time CAC sorption sites will become saturated, resulting in the breakthrough of PFAS. Therefore, it is important to investigate the factors affecting the retention and breakthrough of PFAS in CAC barriers to predict their long-term effectiveness. In this presentation, we will discuss the results obtained from a series of batch adsorption experiments designed to gain insights into the factors affecting the retention of PFAS including 1) the effect of the particle size of the activated carbon; 2) the effect of the competitive sorption among PFAS compounds; 3) the effect of the competitive sorption between PFAS and non-fluorinated co-contaminants such as petroleum hydrocarbons, butyl carbitol, and chlorinated solvents; 4) the effect of the presence of the organic matter; and 5) PFAS adsorption-desorption hysteresis. In addition, the retention and breakthrough of PFAS was investigated using small-scale columns packed with soil and CAC. The data obtained from batch experiments were incorporated into a transport model to investigate the capability of the model to predict the retention and breakthrough of PFAS in the column. Overall, our research provides an improved understanding of PFAS transport and breakthrough in a CAC barrier.

06.04.11 Investigating Salt Marsh Oil Spills and Restoration Tactics

J.L. Ramirez, Unemployed at the moment / Biology; P.L. Pennington, NOAA / National Centers for Coastal Ocean Science

Replanting of intertidal salt marshes with native plant species has been used as an oil spill restoration tactic. These replanting restoration projects attempt to re-establish marsh function and inhibit erosion. Nutrient enrichment may be an option when restoring salt marsh ecosystems but there is conflicting evidence amendment provides an increase in belowground biomass to help stabilize the marsh and prevent erosion. The purpose of this study to determine the level at which marine fuel oil will have an adverse effect on *Spartina alterniflora* and *Spartina patens* and to measure the stem growth and root production of *S. alterniflora* in response to fertilizer treatment. *Spartina sp.* plugs were treated with five different oil slick thicknesses ranging from 0μm to 3000μm in a greenhouse. After 28-d, the plant stem height, stem density, and aboveground and below ground biomass were recorded. Overall, there was no significant effect of oil on plant performance for both grass species. There was no significant difference in stem density and stem height 140-d after removing the standing oiled stem. *S. alterniflora* seedlings were treated with Osmocote™ smart-release fertilizer ("high" and "low" treatments) along with a control. Endpoints included stem density, stem height, aboveground and belowground biomass, root and rhizome length, and root and rhizome diameter. The "high" fertilizer treatment was determined to increase stem density and stem height. There was no significant effect of fertilizer on root or rhizome production. This study focused on plant growth and physiology however, the implications of oil on salt marsh ecosystems at a community and ecosystem level still warrant additional monitoring and research to better understand these interactions.

06.04.12 Seasonality of Sulfur Cycling and Metal Remediation Within Two Constructed Wetlands in the Southeastern United States

C.M. Nicholson, X. Xu, University of Georgia / Savannah River Ecology Laboratory/D.B. Warnell School of Forestry and Natural Resources; R.B. Bringolf, University of Georgia / Interdisciplinary Toxicology Program; A.S. Knox, Savannah River National Laboratory; A. Lindell, University of Georgia / Savannah River Ecology Laboratory; E. Peck, Savannah River Ecology Laboratory

Effective metal remediation in constructed wetlands involves dissimilatory sulfate reduction to produce sulfide and mineralize metals from surface waters. The concentration of sulfide in the sediments is seasonally influenced and has been documented to decrease in the cool season (September–March) in a constructed wetland in the Southeastern U.S. The primary means of metal removal in the cool season shifts to adsorption via organic flocculant, making metals more bioavailable. This change may alter the effectiveness of remediation. This seasonal influence on sulfur cycling in the sediments and macrophytes and its relationship to metal remediation methods have not been explored in a large-scale system. To improve remediation of metals in constructed wetland systems, an observational assessment was conducted considering seasonal sulfur cycling and its association with the location of metal sequestration in two constructed wetlands in South Carolina. The wetland systems differ in maintenance history and age, with one remaining relatively untouched for the past decade, and the other maintained since construction. Both systems were sampled monthly within a warm and cool season to: a) assess the seasonal flux of bioavailable sulfide in the sediments and total sulfur concentrations within macrophyte tissues, b) evaluate if metal concentrations in different sediment layers change seasonally; c) analyze the relationship between increased concentrations of bioavailable metal-fulvic acid complexes and the concentration of metals in macrophyte tissues; and d) explore the influence of age and maintenance on bioavailable sulfide concentrations. The findings of this study will provide management guidance to ensure proper functioning of constructed wetlands remediating metals.

06.04.14 The Development of Diffusive Equilibrium, High-Resolution Passive Samplers for Measuring Perfluoroalkyl Substances (PFAS) in Contaminated Groundwater and Sediments

K. McDermett, A. Jackson, Texas Tech University / Department of Civil, Environmental, & Construction Engineering

Per- and polyfluoroalkyl substances (PFAS) are a large, diverse classification of manmade organic compounds used in numerous industrial and domestic applications around the world, with large quantities being used in oil-resistant food packaging, non-stick coatings, stain repellants, and non-aqueous fire-fighting foams. PFAS are characterized by a strongly bonded carbon-fluorine backbone chain, and can vary in backbone size or functional groups. Following use and disposal, PFAS can be introduced into groundwater and sediments resulting in contamination, which can lead to ecological and human exposures. Once in the subsurface, there is a need to characterize PFAS fate and transport, which can be challenging due to their surfactant nature enabling surface/interface interactions, and the heterogeneity of the saturated media. In order to accomplish this need, passive sampling can be employed. Diffusive equilibrium passive samplers can be directly driven in sediments or groundwater. The samplers equilibrate with pore water through diffusion across the sampler membrane, providing high resolution pore water concentrations of dissolved species. The objectives of this study were to develop a diffusive equilibrium passive sampler to measure PFAS in contaminated groundwater and sediments. To achieve this objective, we first conducted a screening study to determine the membrane mass transfer coefficients, (Km), for a series of PFAS congeners in an ideal system. Based on these results we demonstrated a prototype sampler in a laboratory flow box. Over a deployment period of 21 days, concentrations of both sulfonate and carboxylic PFAS (PFSAs and PFCAs) and some precursors reached equilibrium with porewater (sampler concentration > 90 % of porewater concentration). Application of these samplers could provide improved understanding of the behavior of PFAS in groundwater or sediment systems and allow better measures of ecological exposure. Reliable subsurface site characterization will allow robust site assessments, conceptual models, and improve remediation designs as well as increase confidence in post remedial assessments.

06.04.15 The Journey of Toluene to Complete Mineralization by Heat Activated Persulfate

G. Kalogerakis, H.K. Boparai, University of Toronto / Department of Civil and Mineral Engineering; B.E. Sleep, University of Toronto - Dept of Civil & Mineral Engineering / Department of Civil and Mineral Engineering

Groundwater contamination by petroleum hydrocarbons (PHCs) is a widespread and persistent problem. The most common contamination source is underground leaking storage tanks. Due to the presence of buildings and other structures at such sites, *in situ* remediation is preferred over the *ex situ* methods. I*n situ* chemical oxidation (ISCO), involving persulfate activated by different methods, is a promising remediation technology for the PHCs such as toluene and benzene. Past research has shown the removal of parent compounds by activated persulfate but complete mineralization, via evolution of CO_2, has not been monitored. Moreover, the gaseous by-products have been observed but the formation of solid intermediates is seldom reported.Our study has thoroughly examined the mineralization of toluene by heat activated persulfate in batch experiments. The effect of temperature and oxidant dose on the degradation of toluene is investigated. The fate of several intermediate products and the consumption of persulfate up to complete toluene mineralization are monitored. Further, we report on the evolution of gaseous products as a result of toluene mineralization and oxidant decomposition.The results of this investigation advance our understanding of the time scale needed for the complete mineralization of toluene as well as the optimal oxidant dose and temperature. Formation of gaseous and solid products may block the soil pores and reduce hydraulic conductivity, possibly hindering the contact between the oxidant and contaminant. Thus, minimizing the formation of the gaseous and solid products should be considered for developing an efficient ISCO remediation design.

06.04.16 Treating Agricultural Runoff with a Mobile Carbon Filtration Unit

B.M. Phillips, University of California, Davis / Environmental Toxicology; L.B. McCalla, University of California, Davis, Marine Pollution Studies Laboratory at Granite Canyon / Environmental Toxicology; C. Siegler, University of California, Davis / Environmental Toxicology; R. Clark, J. Adelaars, Central Coast Wetlands Group; X. Deng, California Environmental Protection Agency / Pesticide Regulation; R.S. Tjeerdema, University of California, Davis / Environmental Toxicology

Much of California's intensive vegetable production, valued at over $7 billion annually, occurs in the Central Coast region of the state. Several classes of pesticides used have been shown to impair water quality in California, including organophosphates, pyrethroids and neonicotinoids. Vegetative treatment systems (VTS) can reduce pesticide loads and associated toxicity in agricultural runoff, but many water soluble pesticides such as neonicotinoids are not effectively treated by VTS. Recent studies have shown that activated carbon or biochar filtration can be used to remove soluble contaminants, especially when coupled with other VTS components. Although effective at reducing contaminant concentrations and toxicity, installation of a VTS is not always an option for growers required to remove non-crop vegetation for food safety concerns. A mobile carbon filtration treatment system, either offered as a rented service or as a purchased product, could be used in lieu of a permanent vegetated installation. We evaluated a filter system consisting of a trailer-mounted tank containing 600L of biochar. Input water from a 1080 acre agricultural drainage was pre-filtered with 0.25-mm sand and 0.1-mm particle filters. 1.5 million liters of water were treated during two study periods (9 and 12 weeks). Laboratory toxicity tests, and chemical and nutrient analyses were conducted on input and output water every three weeks. A range of 11 to 21 compounds were detected in each sampling period. Pesticide concentrations were reduced by greater than 99% at the first sampling period. Efficacy declined linearly in subsequent periods. Based on declining curves, biochar was expected to remain at least 50% effective for up to 34 weeks. Toxicity was assessed with *Ceriodaphnia dubia*, *Hyalella azteca* and *Chironomus dilutus*. Treatment success was based on the sensitivity of the organism. Significant input toxicity was reduced to non-toxic levels in 6 of 16 samples, but was significantly treated in 5 additional samples. Some input concentrations of the neonicotinoid imidacloprid and the pyrethroid cypermethrin exceeded organism-specific toxicity thresholds and benchmarks, but the overall causes of toxicity were complex mixtures of agricultural chemicals. Nutrients were not significantly reduced by the biochar. These results demonstrate the utility of biochar in treating agricultural runoff, and provide measures of the longevity of biochar under field conditions.

06.04.17 Utilization of Biochars to Remediate Mercury and Methylmercury Contaminated Soils on the Savannah River Site

B. Jensen, University of Georgia / Savannah River Ecology Laboratory; X. Xu, University of Georgia / Savannah River Ecology Laboratory/D.B. Warnell School of Forestry and Natural Resources; R.B. Bringolf, University of Georgia / Interdisciplinary Toxicology Program

Mercury (Hg) continues to be a threat to the environment and ecosystems through anthropogenic sources, resulting in high concentrations of the contaminant in both the sediment, water, and trophic webs. In high concentrations, Hg can be harmful to both terrestrial and aquatic wildlife, as well as humans through consumption of wildlife. Remediation using soil amendments, or organic additives, is a unique technique that can be applied at low cost and low impact to both terrestrial and aquatic ecosystems that contain or receive high concentrations of Hg as well as other metals. Of interest is the Savannah River Swamp System within the Savannah River Site (SRS), which previously received high concentrations of Hg contamination from the Olin Corporation Chlor-Alkali Plant. This system experiences annual periodic flooding, which provides anaerobic conditions for methylation of Hg in soils of the floodplains. These anaerobic conditions will be simulated in a mesocosm study with soil amendments serving as treatments to bind and demobilize Hg. The focus of this research will be on the remedial effectiveness of biochars: sulfur-modified and unmodified rice husk and peanut hull. The primary goal of this research will be to determine whether these soil amendments are an effective method for remediating Hg from soils with high concentrations of this metal. Determining the effectiveness of these amendments will provide an insight into the applicability of this type of remediation in similar sites that either experience legacy or continued contamination of Hg in the environment.

06.20 Late Breaking Science: Engineering, Remediation and Restoration

06.20.02 State of the Environment: Occurrence and Distribution of Neonicotinoids, Pharmaceuticals, Per - and Polyfluoroalkyl Substances in the Environment

S.T. Kurwadkar, California State University Fullerton / Civil and Environmental Engineering

Occurrence and distribution of various legacy and emerging contaminants of concern, including neonicotinoids, pharmaceuticals, and per- polyfluorinated substances (PFAS), in multimedia environments, is often reported with increasing frequency. Their spatial and temporal distribution across different environmental media is a cause of concern. Recently, many studies often focused on the regional aspect, emphasizing the specific contaminant and media and without any context to evolving global response to manage and mitigate the occurrence and potential human health and ecological impact. Global response including, the development of alternative substances, gradual phasing out their use, and even banning or restricting their use only for specialty purposes, are some of the documented responses worldwide. Additionally, global regulatory responses have rarely been discussed, such as establishing the lifetime health advisory limits, setting up environmental quality standards, creating contaminant candidate lists, listing them as persistent organic pollutants, and re-evaluating human health and ecological risks. This article presents a comprehensive discussion on a global scale, emphasizing some of the hotly debated environmental contaminants – neonicotinoids, pharmaceuticals, and PFAS. A global perspective is presented with emphasis on documented instances of occurrence of these compounds and global regulatory response to manage and mitigate human health and ecological risk arising from exposure to these compounds through various routes and exposure pathways.

07.01 Communication Programs and Guidelines: Let's Take Action

07.01.02 Learning from Climate Change Communication to Expand and Improve Nutrient Communication

K. Canfield, USEPA / Social Sciences; K. Mulvaney, U.S. Environmental Protection Agency / Atlantic Coastal Environmental Sciences Division (ACESD); N. Merrill, USEPA / Social Sciences

Communicating about environmental problems with "slow impacts" has long been a challenge for scientists, public health officials, and science communicators, as a time delay between the problematic action and subsequent consequences dilute the sense of urgency to act. This is an important challenge for both nutrient and climate change communication. As nutrient pollution continues in marine waters in the United States and globally and impacts worsen, the need for effective nutrient communication is increasing. We hypothesized that the findings from research on climate change communication could provide an important set of evidence-backed practices that could be applied toward improving nutrient communication to tackle these worsening impacts. Using a qualitative coding approach to review the science communication and climate change communication literatures, we identified five recommended practices for climate change communication that are appropriate to transfer to nutrient communication: 1. prioritize two-way communication between the public and communicators, 2. relate to human experience rather than abstract analysis, 3. emphasize local impacts and immediate actions to be taken, 4. define and activate social norms around the problem and urgency of action, and 5. build interdisciplinary collaborations to address science communication training and recognition gaps. These practices share an underlying emphasis on relating the communication to the societal and environmental context and recognizing the assets of all relevant publics and individuals to address the environmental challenge. Recognizing the differences between climate change and impacts of nutrient pollution, we also explore how these environmental problems with delayed impacts demand nuanced strategies for more effective communication and public engagement. Using examples from our own efforts to communicate about excess nutrients on Cape Cod, Massachusetts, we demonstrate how these practices can be applied in navigating the communication of slow impacts in a diversity of settings, including across government agencies. Applying generalizable approaches to successfully communicate about the slow impacts related to nutrient pollution across geographic contexts will help build publics' understanding and urgency to act on comprehensive management of nutrient pollution, thereby increasing protection of coastal and marine environments.

07.01.03 How to Assess Effective Science-Based Risk Communication?

D. Bedolla López, Egesta Lab / Institute for Resources, Environment and Sustainability; G. Oberg, University of British Columbia / IRES; A. Leopold, Calidris Environment BV

The efficiency of science communication training is increasingly questioned. There are presently no commonly available tools that allow instructors and participants to assess whether or not the training led to stronger communication skills. The only tools commonly available are self-report surveys, which are poor quality-indicators. The aim of this study is to identify factors that shape effective risk communication and to develop a tool to assess the effectiveness of science communication training related to chemical risk. In collaboration with the SETAC interest group SCIRIC, this study characterizes weaknesses of current training programs, designs a beta version of an evaluation tool and tests its utility in a science communication workshop organized by SETAC Africa. The study finds that a focus on strengthening journalistic skills and one-way, clear, and concise presentation of facts — a common practice in current science communication training — is insufficient and can be counter-productive. The beta-version of the evaluation tool shows promise in assessing participants' awareness of the advantages of dialogue-based communication. A different approach to science communication training is needed, and the beta-version of the tool developed in this study has the potential of assessing the training performance of a two-way communication strategy, which is expected to improve scientists' communication skills. Further development is needed to identify and foster ways to operationalize and measure whether or not training improves scientists' ability to design, select and utilize strategies leading to more effective science risk communication.

07.01.04 Above and Beyond the HQ: Best Practices for Summarizing Ecological Risk Results

R. Zajac-Fay, Geosyntec; J. Arblaster, J. Zodrow, J.M. Conder, Geosyntec Consultants

Communicating conclusions of ecological risk assessments (ERAs) can be challenging. Non-risk assessors often find risk assessment details confusing, and they often only read the final conclusion of ERAs. It is imperative to communicate ERA conclusions clearly and effectively, as: 1) they will be read by multiple audiences with different backgrounds; 2) they are fundamental to the regulatory process and guide decision making at contaminated sites; 3) stakeholder acceptance of decisions require that they understand the basis of recommendations; 4) there is the potential that they will be used in litigation proceedings (requiring precise language); and 5) a non-risk assessor may be responsible for communicating the ERA results. The hazard quotient (HQ) determined during an ERA is often used to communicate risk. Although there can be advantages to results being presented in a simplified manner, this simplicity can also cause confusion and misinterpretation of the results. For example, an HQ greater than one does not necessarily imply adverse effects of risk, but this is a common misinterpretation. There are better ways to communicate the results of ERAs. This presentation will include real examples of language common in ERA conclusions based on HQs, discuss the issues and potential confusion with HQs, and provide recommendations on how to summarize your results into one meaningful summary sentence that goes above and beyond the hazard quotient and will be the *pièce de resistance* of your ERA.

07.02 Contaminants of Emerging Concern and Superfund: What Lies Ahead

07.02.01 Emerging Contaminants and the Law (Right Now)

S. Bell, Farella Braun Martel

Contaminants of emerging concern – particularly perfluorinated compounds – are the subject of continued focus at the state level (in certain states), and renewed focus at the federal level. This panel will provide an overview on the federal and state regulatory framework regarding PFCs, an update on the status of the U.S. Environmental Protection Agency's 2019 PFAS Action Plan, and a legal perspective on the potential listing of certain per- and polyfluoroalkyl substances (PFAS) as "hazardous substances" under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). The addition of certain PFAS to the "hazardous substances" designation under CERCLA has the potential to result in new litigation and "reopening" investigations and/or litigation involving previously remediated Superfund sites. This panel will also provide a legal perspective on these implications, as well as trends in litigation relating to PFCs, and a look at what is ahead regarding other emerging contaminants and the law.

07.02.02 Regulating Emerging Contaminants as a Single Class: Does this Make Sense?

K. Robrock, G. Caviness, Exponent, Inc.

Some emerging contaminants, such as halogenated flame retardants and per- and polyfluoroalkyl substances (PFAS), are classes of chemicals comprised of hundreds or thousands of individual chemicals. In recent years, some organizations have argued that all of the individual chemicals within a given class are sufficiently similar as to be regulated as a single class. Legislators in some states have followed suit by considering or implementing restrictions applicable to large classes of chemicals in certain consumer products. For example, the state of California restricted the use of flame retardants in certain household furnishings and children's products, and Vermont recently banned enacted legislation that will ban PFAS and other classes of chemicals in a number of consumer products. While restrictions are intended to protect human health, they are based on the underlying assumption that chemicals that have a chemical moiety in common are similarly hazardous. However, as has been acknowledged by the National Academy of Sciences, there is little scientific basis for large groupings of hundreds or thousands of structurally dissimilar chemicals, which will consequently have different environmental and toxicological properties, as a single class. This paper discusses the scientific basis for past regulatory efforts for chemical groupings and mixtures (e.g., dioxins, polychlorinated biphenyls, polyaromatic hydrocarbons, petroleum hydrocarbons) and presents cautionary considerations for grouping and regulating hundreds to thousands of chemicals as a single class for regulatory purposes.

07.02.03 Lessons from Addressing Last Century's Contaminants of Emerging Concern: Radioactive Waste Cleanups After the Manhattan Project

K.G. Keil, U.S. Army Corps of Engineers / ERDC; V.M. Harms, L.S. Berta, U. S. Army Corps of Engineers

Advances in analytical techniques and a better understanding of ecological and human health risks drive changes to regulatory and investigative frameworks for contaminants of emerging concern. This is exactly what happened as our nation began addressing the legacy of radioactive waste resulting from development of our first atomic weapons at the onset of World War II. Over the last several decades we have spent billions of dollars addressing the environmental impacts of radioactive waste sites. Lessons learned from these cleanups may be applied to addressing this century's emerging contaminants. There are several parallels which may be drawn regarding how we've addressed these now legacy contaminants and how we are beginning to address emerging contaminants. For example, radiation protection standards addressing both environmental and human health have evolved significantly since radiation was first discovered at the turn of the 20th century. The regulations addressing PFAS seem to be following a similar pattern of evolution thus far. In this presentation, case studies from sites contaminated with radioactivity after supporting the Manhattan Project will be provided. At some of these sites, advances in radiological analytical techniques coupled with a more thorough understanding of both fate and effects of radioactivity in the environment resulted in additional, broader investigations and ultimately more comprehensive remedial actions. Radioisotopes which were not initially identified or quantified were later found to contribute significantly to environmental impacts and ultimately drove remedial efforts at some sites. Are our analytical techniques for detecting PFAS and 6-PPD keeping up with our understanding of how these compounds behave in and effect the environment? And are our regulations keeping up with their release to the environment? Or are we still in a "Manhattan Project" (war time) mind set of secrecy and haste when dealing with the release of emerging contaminants to the environment? That type of mindset ultimately led to an immense legacy of radioactive waste which we will continue to address for many more years to come.

07.02.04 Source Identification and Management of PFAS in Stormwater

J. Pietari, J. Wilkinson, E. Wood, Ramboll / Environment & Health

PFAS concentrations in groundwater exceeded state regulatory thresholds at an industrial facility that has spray-applied polytetrafluoroethylene (PTFE) and other fluoropolymer coatings onto medical devices for over 40 years. The facility is located in a groundwater resource area where the groundwater is used by nearby public and private water supply wells. Stormwater runoff from the facility roof was identified as a primary source of PFAS in the groundwater. Initial assessment indicated that three primary sources contributed to PFAS in the roof runoff including historical PFAS air emissions; roof materials impacted by the air emissions; and the release of solid pieces of PFAS-impacted residue agglomerated in facility spray booth stacks and bake oven stacks. Up to 1,264 ng/L of PFAS were detected in the roof runoff. Initial mitigation activities included replacement of the impacted roof and cleaning of the stacks; however, PFAS concentrations remained elevated in the roof runoff. Further investigation was needed to determine the sources of PFAS in roof runoff with more specificity. Initial hypotheses included several pieces of equipment on the roof in addition to air emissions. Comprehensive sampling of the roof equipment, including spray booth and oven stacks, vents, and air handling equipment, in addition to residues accumulated on the new roof was conducted to further identify and possibly eliminate the sources of PFAS-impacted roof runoff. A second objective of this work was to determine an acceptable concentration of PFAS in the roof runoff that would be protective of the groundwater resource and meet regulatory thresholds. A case study will be presented that illustrates:the multiple complexities addressed in identifying the sources of PFAS in stormwater at this industrial facility,development of an algorithm to prioritize identified sources for removal and/or replacement to further reduce PFAS concentrations in roof runoff, andhow the established threshold will be used to monitor the effectiveness f the proposed source removal activities.

07.02.05 Land Application of Biosolids: Effective Management with Focus on Emerging Contaminants

W.L. Goodfellow, M.W. Kierski, B. Drollette, G.J. Getzinger, Exponent, Inc. / Environmental and Earth Sciences

Federal biosolid rulemaking efforts in the United States began in the mid-1970s. The USEPA published "The Standards for the Use or Disposal of Sewage Sludge" (Title 40 of the Code of Federal Regulations, Part 503), which became effective March 22, 1993. This regulation is often referred as "The 503 Rule". This rule established requirements for the final use or disposal of sewage sludge (often termed biosolids) when they are: 1) applied to land to condition the soil or fertilize crops or other vegetation growth in the soil; 2) placed on a surface site for final disposal; or 3) fired in a biosolid incinerator. State regulations also apply to biosolids use or disposal and may be subject to local requirements as well. The 503 Rule is based on an extensive multimedia risk assessment. The research results and management experience addressed 25 potential pollutants using 14 exposure pathways in the risk assessment. This paper will focus on the land application of biosolids and the growing challenges in effectively managing their application to address emerging contaminants. Per- and polyfluoroalkyl substances (PFAS) in the environment have been the subject of recent public and regulatory scrutiny. Our paper will explore the current requirements with regards to biosolids management, using PFAS as an example, to describe the challenges surrounding land application practices. We will also discuss various state policies that have recently been released addressing land application of biosolids and how they line-up with regards to current data on human health and ecological risk. The objective of our paper is to provide the necessary risk-based context to effectively explore different biosolids management practices including the use of land application methods.

07.02.06 Screening Assessment Framework to Assign Relative Risk to PFAS: An Approach to Understand and Assess PFAS Mixtures

D. McCue, EHS Support; C. Mancini, EHS-Support / Contaminated Sediment; N. Goulding, EHS Support LLC; A. Miano, EHS Support

More than 9,000 per- and polyfluoroalkyl substances (PFAS) have been identified by the U.S. Environmental Protection Agency (USEPA) with most lacking toxicological data. As PFAS analytical methods continue to improve in their capability to address this growing list, regulatory programs across the United States and abroad have used read-across extrapolations from one PFAS compound to another to establish regulatory standards and screening criteria. The use of simple similarity evaluations where toxicity or potency is determined proportional to carbon chain length is prone to overestimating the potential for risk. Further, based on the current state-of-the-science, other methods to assign toxicity, such as additivity and relative potency factors, have also been found by others to be inappropriate to estimate risk. To address these limitations, a tiered screening assessment framework has been developed that leverages approaches currently used in the United States and internationally for the assessment of industrial chemicals along with approaches currently used in traditional human health and ecological risk assessments conducted under USEPA's Superfund Program paradigm. The goal of this framework is to categorize or assign PFAS compounds into tiers (low, medium, high) based on their relative risk. Specifically, this categorization approach considers the following elements: chemical classification (functional group, polymer or non-polymer); human and ecological toxicity data (adverse outcomes, potency, elimination half-lives); environmental persistence and occurrence, including the presence of common mixtures observed in environmental media; bioaccumulation/biomagnification; and, site-specific information (receptors, sources, regulatory interest). In addition, an uncertainty analysis is conducted with the impact of the uncertainty incorporated into the assessment of relative risks. Validation of this approach against a range of PFAS compounds measured in environmental media from site-specific data sets has been conducted. Completion of the study has resulted in the classification of PFAS compounds into tiers which more accurately represent relative toxicity and clearly highlights major bias introduced using simplistic read-across methods. The results have demonstrated the feasibility and flexibility of this framework for use in Superfund, in particular for potentially responsible party (PRP) cost allocation and risk communication/risk management.

07.03 Environmental Justice: Methodologies to Incorporate Environmental Justice into Human Health Risk Assessment

07.03.01 Quantitative Methods for Environmental Justice in Human Risk Assessment: An Overview

W. Rish, A. Verwiel, ToxStrategies, Inc

Executive Order 14008 signed on January 27, 2021 has established environmental justice (EJ) as a core priority of the Biden Administration. There is a need to enhance quantitative risk assessment methods to be more representative of EJ community exposures and risks. It is critical that improvements be made such that exposures to chemical stressors, non-chemical stressors, and their interaction with dose response in EJ communities are properly characterized. Based on an extensive literature review, the authors present an overview of the state of science for incorporating EJ factors into quantitative human health risk assessments of environmental chemical exposures. General methodological and data needs are identified. This presentation is meant to set the stage for the session.

07.03.02 Guidelines for Cumulative Risk Assessment Planning and Problem Formulation

L. Martin, USEPA

USEPA drafted Guidelines for Cumulative Risk Assessment Planning and Problem Formulation. The Guidelines lay the foundation for considering current and anticipated future cumulative risk analytical methods and provides guidelines for when such assessments are appropriate. They provide recommendations for developing a CRA analysis plan and are intended for use with other EPA guidelines on methods such as the *Guidelines and Supplementary Guidance for Assessment of Chemical Mixtures*. A defining feature of CRA is the methodological combining, or addition, of multiple stressors that can result in an adverse health outcome. Another important characteristic is that the CRA problem formulation can focus on either the stressor or the receptor. A CRA problem formulation beginning with adverse outcomes on a receptor (e.g., tissue, organism, community), seeks to examine the stressor(s) causing the adverse outcome. This is of particular value to communities expressing environmental justice concerns. Multiple agents or stressors can be interpreted broadly to include chemical mixtures, combinations of chemicals that share a common mode or mechanism of action or adverse outcome, chemical and nonchemical factors that might interact, or any combination of the above linked in analysis through the CRA problem formulation. This is responsive to concerns that disadvantaged human populations may be more vulnerable to regulated stressors as a result of psychosocial exposure-response modifiers. Different frameworks and methods for analyzing combined multiple stressors are discussed. CRA follows the risk assessment convention of examining toxicological dose-response effects on adverse outcomes from common mechanisms of toxicity, common key events, or converging adverse outcome pathways, and is distinguished from cumulative impact or health impact assessments which may consider a wider range of circumstances. Determining the appropriate assessment method requires clarity on the decision to be made and the scientific questions that need to be answered. These Guidelines describe considerations for when CRA is a suitable assessment method and detail steps for planning the CRA to meet the need of the risk manager. CRA can be applied to human health, ecological, or integrated outcomes.

07.03.03 A Cumulative Air Pollution Risk Model for the State of Minnesota (MNRISKS): Investigating Environmental Justice and Source Prioritization

K. Ellickson, D. Kvale, Minnesota Pollution Control Agency / Environmental Analysis and Outcomes; J. The', Lakes Environmental Software

Identifying potential human health risks from air pollution involves the integration of air emissions inventories, air dispersion and deposition modeling, fate and transport modeling, and oral and inhalation toxicity values. The results of this type of modeling are spatially refined estimates of potential cumulative human health impacts from air pollution. The MNRISKS model is developed every three years from the Minnesota Air Emissions Inventory for both point and non-point sources. Point source emissions are available at the stack level, and non-point emissions are available at the county level. The county level non-point emissions are further processed and allocated into census block groups using surrogate data for more spatially refined outputs. The Minnesota Pollution Control Agency provides processed emissions, source parameters, meteorological data, a receptor grid, and building files to Lakes Environmental Software who incorporate these data into an air dispersion model (AERMOD) and the EPA Human Health Risk Assessment Protocol. The results of this modeling are air concentrations, media, food product concentrations and potential human health risks. We present and compare these risk results by source types, groups of chemicals, health endpoints, and the demographics of nearby populations. These results, together with environmental justice metrics, are used to prioritize small business grants, compliance and enforcement audits, and non-point emissions reduction work. The highest potential contributors to cumulative air pollution risks or chemicals of concern are variable throughout the state, and therefore statewide means and summaries are not informative for the purpose of environmental justice.

07.03.04 Exposure Monitoring Towards Environmental Justice

L. Racz, ToxStrategies, Inc

Executive Order 12898, signed on January 27, 2021, established environmental justice (EJ) as a core priority of the Biden Administration. One factor related to ensuring environmental justice is accurate characterization of community exposures to chemical stressors. Enhanced exposure monitoring approaches that better provide for EJ community involvement and that are more representative of local exposures are needed. Recent advancements in environmental monitoring with personal and portable sensors, especially when deployed using community partnerships, can capture chemical exposures with sufficient resolution to characterize exposures down to the neighborhood level. Use of internet-linked sensors will also require thoughtful advances in management of big data to inform meaningful and time-sensitive decisions. This presentation will cover the state of the science in these sensors and offer insight into how they have been and may be used successfully to advance EJ goals.

07.03.05 One Size Does Not Fit All: Epidemiological Data and Methods to Protect Vulnerable Groups

M. Fox, Johns Hopkins Bloomberg School of Public Health / Health Policy and Management; R. Chari, Rand Corporation; M. Sheehan, Johns Hopkins Bloomberg School of Public Health / Health Policy and Management; A. Chiger, Johns Hopkins Bloomberg School of Public Health / Environmental Health and Engineering; S. Baksh, Johns Hopkins Bloomberg School of Public Health / Department of Epidemiology; K. Nachman, Johns Hopkins Bloomberg School of Public Health / Environmental Health and Engineering

For more than twenty years, the concept of cumulative risk assessment has offered the promise of "holistic reduction of risk", with the acknowledgement that environmental exposures are complex including chemical and non-chemical stressors. Since EPA's 1997 Guidance on Cumulative Risk Assessment called out research, practice and methodological needs, some progress in research and practice has been made while methods are largely stagnant. Building on a limited foundation of existing risk methods that account for vulnerable populations and chemical and non-chemical stressors, additional examples based in epidemiology and systematic review are presented. Previous work has examined: 1) vulnerability due to socio-economic status on the IQ effect of the Pb NAAQS; and 2) susceptibility due to pre-existing disease on neurodevelopmental effects of MeHg. An approach under development will examine ways to incorporate the community health context in site-specific assessments. These examples illustrate evidence-based methods to develop susceptibility- and vulnerability-informed risk metrics, advancing risk assessment tools and the effort toward environmental justice.

07.03.06 The Importance of the Use of Exposure Factors for Indigenous Communities Within Risk Assessments

S. Fernandes, H. Phillips, L. Leon, CanNorth

Risk assessments that consider Indigenous communities must integrate relevant information into all aspects of the exposure assessment. The frequency and consumption rates of country foods can vary widely depending on the geographical location, species available, and traditional values and practices of a community. Therefore, the information must reflect the local community and site-specific considerations. Without obtaining site-specific consumption rates and concentrations, risk assessments must rely on generic information that may not reflect the traditional foods within an Indigenous community. Other factors that may not rely on site-specific information, such as soil ingestion rates while participating in harvesting activities, can also be considered in a risk assessment. Additional information is needed on these types of factors.An example of incorporating Indigenous exposure values will be discussed based on a risk assessment that was conducted to support the Giant Mine Remediation Plan in the Northwest Territories, Canada. A significant part of the human health risk assessment (HHRA) involved community engagement with the Yellowknives Dene First Nation as well as the North Slave Métis Alliance. A dietary survey instrument was designed to collect information on how much country foods people in these communities ate in order to reduce uncertainties in the HHRA. Traditional knowledge was shared during these dietary survey activities. In addition, a voluntary country food sample program was initiated to collect country food samples from the community to represent the different types of food people ate. It was important that the risk assessment reflected the types of foods that were consumed locally, as well as where they would be collected. Another aspect that was integrated into the exposure assessment was the parts of the animal that would be consumed. For example, for fish, samples were analyzed for fillet, eyes, and the fat layer under the skin as the dietary survey revealed that these are all important components to the local Indigenous communities. Overall, these types of risk assessments need to respect how the local Indigenous communities use the land and their hunting and fishing areas. The assessments need to start moving away from a pure Western Science Approach and be more mindful of land stewardship, cultural and spiritual considerations in their design.

07.03.07 Evaluating the Vulnerability of the Pueblo De San Ildefonso to the Combined Effects of Extreme Events and Contaminant Exposure

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The members of the Pueblo de San Ildefonso (Pueblo) face significant environmental justice challenges due to their proximity to the Los Alamos National Lab (LANL) in New Mexico, and changing climate conditions. The Pueblo is facing changing climate conditions, including increasing average temperatures as well as more frequent and/or intense incidences of extreme heat, drought, wildfires, storms, and floods, typical of the US Southwest region. These changing climate conditions are expected to have a negative impact on human health (e.g., on respiratory and cardiovascular systems, heatstroke, hyperthermia, and death). At the same time, the Pueblo also faces potential human health impacts due to its proximity to the LANL. Historical Lab activities have left a legacy of plutonium and other radionuclides in the local environment. Extreme events such as wildfires, extreme rainfall, and flooding may increase the risk of mobilization and exposure to contaminants on the Pueblo from the Lab. In this paper we present a cumulative health impact assessment to understand the vulnerability of the members of the Pueblo to the direct, indirect, and combined effects of climate change by characterizing risks from non-chemical and chemical stressors. First, we evaluate the direct health impacts associated with climate change. We develop unique climate load health indices for two key climate hazards (heat and wildfire). Higher climate load indices indicate a higher likelihood of negative climate-related health effects for members of the Pueblo in the future. We projected that the overall climate load for the Pueblo is worse in the future compared to present day conditions. The climate load can be used as a proxy for understating the severity of climate-related health impacts in the future. Then, we characterize the indirect health impacts of climate change, focusing on the potential for extreme events to increase contaminant exposure and human health risks, through an exemplar case study (2013 flood event). We found that excess cancer risk was greater with post-flood surface water and sediment exposures compared to pre-flood surface water and sediment exposures. Finally, we qualitatively discuss the combined effects of direct and indirect climate change impacts and contamination on human health, through a review of proxy literature. We found that pollutants and climate stressors may interact and cause more harm together than on their own.

07.04 Importance of Science-based Research in Improving Human Health and the Environment

07.04.01 De Minimus, Harvey Washington Wiley and the 1906 Pure Food and Drug Act

A.S. Kolok, University of Idaho / Fish and Wildlife Science

The concept that dose defines a poison harkens back to Paracelcus, a sixteenth century Swiss physician. A corollary of the concept is *de minimus*, the idea that potentially toxic chemicals, when used at very low levels, are non-toxic. When viewed through a modern lens, it is tempting to believe that the concept of *de minimus* only fell into disfavor recently, when innovations in molecular biology, endocrinology, and analytic chemistry, substantiated that low dose exposures were initiators of carcinogenesis and endocrine disruption. This, however, is not the case, for the history of questioning *de mimimus* can be traced back over 120 years to Harvey Washington Wiley. Wiley is best known as the champion of the 1906 Pure Food and Drug Act, as well as the organizer of the Poison squad, a group of 12 men (all Federal employees!) that volunteered to consume meals laced with a one of several different chemical preservatives. In his 'hygenic table trials', Wiley meticulously prescribed individual preservatives to the men in low doses, which invariably resulted in mild to acute digestive distress. When discussing his results in subsequent published bulletins, he directly questioned *de minimus*, specifically related to chemicals mixtures. He rationalized that the diets of many American urbanites contained preservative mixtures and that these chemicals were acting synergistically on the kidney's capacity for excretion, potentially resulting in irreparable kidney damage. Wiley was a staunch advocate for open communication, both professionally and through the popular press, and despite that fact that his intentions were pure, his communications opened him up to considerable criticism, including inaccurate interpretation of his work and personal attacks. Indeed, the passage of the 1906 Pure Food and Drug Act would not have occurred, despite all of Wiley's efforts, had it not been for synergies outside of his professional control. Wiley's struggles with *de minimus* and the passing of the 1906 Act have corollaries today, particularly as they pertain to the interface between scientific inquiry and public policy. Using scientific understanding to inform public policy is challenging and potentially subjects scientists to public and political criticism. Nevertheless, it is just as necessary today as it was 120 year ago, when Harvey Wiley conducted research regarding food safety and the toxicological concept of *de minimus*.

07.04.03 An Expanded Role for the Risk, Toxicological, and Environmental Sciences in Addressing Pandemics

K. Grasman, Calvin University / Biology

Clinical medicine, public health, immunology, and microbiology have been at the forefront of scientific research, policy formulation, and public education addressing the COVID-19 pandemic. While often overlapping these fields, the disciplines of risk assessment, toxicology, disease ecology, One Health, and more broadly environmental science have tools and perspectives that can contribute uniquely to pandemic responses. Many businesses and community organizations with little experience making science-based decisions have had to do so during the pandemic. Risk scientists (e.g., SETAC) frequently assist governments and businesses with science-based decisions, including quantitative assessment and monitoring and use of metrics for making specific decisions. Although many public health and government officials have proposed to use such metrices for managing the pandemic, in actual practice these criteria often have been ignored. Toxicologists understand the risks of invisible chemicals, similar to those of microbes, and the biomagnification of chemicals through food chains, similar to the exponential growth of a virus in an immunologically naïve population. Citizens and organizational leaders often do not understand how actions of individuals or small groups add up to a high level of population risk when large numbers of people or organizations act in the same way. Risk assessors can help individuals and organizations understand these cumulative risks. Environmental scientists have expertise dealing with scientific uncertainties and controversies and debunking pseudoscience used to support predetermined business or policy positions. Toxicology and disease ecology are foundational areas of One Health, and as a zoonotic disease with environmental dimensions, at its basis COVID-19 is essentially a One Health issue. Finally, environmental scientists understand the "tragedy of the commons"—the overuse of scarce, commonly held resources. During the pandemic individuals and organizations have clamored for their "share" of "activity levels," which are essentially scarce common resources, resulting in a total amount of interpersonal interactions that exceed the levels necessary for controlling viral spread. Going forward risk science societies such as SETAC should work towards the incorporation of these tools and perspectives into government policies and business practices in more formal ways to enhance public health approaches whose success has been limited.

07.04.04 An Era of Misinformation

L. Kapustka, LK Consultancy; R.G. Stahl, DuPont (retired)

The COVID-19 experience of 2019-21 exposed many cracks in our social structure, not the least of them being misinformation and disinformation that likely exacerbated and prolonged the pandemic. Conspiracy tropes ricocheted across social media platforms casting doubts in some about the seriousness of the disease, its transmissibility, protective measures, and ultimately vaccine effectiveness. As challenging as it has been to witness the spread of erroneous information, sadly, this is not new. Human societies have experienced theocratic conflicts for ages. Poignant examples include ovists v. spermists; geocentrism and flat-eartherism versus heliocentrism; and more contemporaneously, creationism versus evolution. There is apparent comfort for many to be able to embrace simplistic explanations that offer a sense of certainty instead of embracing the self-correcting constructs of science. After touching on these examples, I will conclude with a timeline showing the science and rationale regarding wearing masks as new data informed the changing recommendations. This compressed timeline of approximately one year will be used to illustrate longer histories that track changes in science-based understanding of how the natural world works.

07.04.05 Filtration Efficiency, Breathability and Flow Resistance of Barrier Face Coverings and Common Home Fabrics Used for Face Masks During the COVID-19 Pandemic

J. Ayodeji, Texas Tech University / The Department of Environmental Toxicology; M. Khyum, S. Ramkumar, Texas Tech University / Department of Environmental Toxicology

During the outbreak of the COVID-19 pandemic, wearing face coverings was one of the major mitigation measures to protect public health. This followed recommendations from the WHO and the U.S. Centers for Disease Control that barrier face coverings protect both the wearer and others from infection through droplets and aerosols transmission. The resulting increase in demand caused a global shortage of protective face masks. Because aerosols and droplets can be removed by fabric fibers through a series of filtration mechanisms, homemade masks were recommended as alternatives in situation where face masks are not available. However, their filtration performances have not been fully evaluated. In this study, test methods in the ASTM standard (F3502-21) were used to assess particle-size dependent filtration efficiencies (FE) and breathability (pressure drop, Δp) of face coverings and home fabric materials (16 types, 2-ply). Submicron particulates (d = 0.03, 0.06, 0.08, 0.1, 0.2 and 0.3 μm) FE was measured by particle transmission through samples (n = 4) using Condensation Particle Counter (CPC model 3771) equipped with differential mobility analyzer (DMA, model 308100) and aerosol neutralizer (model 3077, all TSI Inc.). Manually generated droplets of a suspension of 0.1 μm-diameter fluorescent nanoparticles in distilled water, to mimic the size of SARS-CoV-2 virus (0.07 - 0.10 μm diameter), was passed through the samples to evaluate flow resistance (FR). Flow was determined by measuring fluorescence (excitation, 580 nm; emission, 605 nm) of beads residual collected at reverse side of samples using a Synergy H4Multi-Mode Microplate Reader (BioTek Instruments, Winooski, VT). Results showed that the FE (15 – 93%) and Δp (0.8 – 71 mmHg) varied considerably among fabrics. Of the total mask types, 18.75% and 81.25% met the minimum FE and breathability standards, respectively, in the ASTM F3502-21 for the tested particulates. Performance (qF) was highest for Velcro style mask (max qF = 3.36, d = 0.03; min qF = 2.80, d = 0.2) and lowest for Dutch wax print fabric (max qF = 0.12, d = 0.1; min qF = 0.04, d = 0.3). Low flow resistance was observed in bandana, neck gaiter, t-shirt I, tank top and bedspread fabrics. Fabric characteristics (composition, thickness, density and waxing) seems to affect performance. Since breathability decreases with increasing FE, evaluation of masking materials serves as crucial guide for the design of optimum barrier face coverings.

07.04.07 Bridging One Health and the Exposome

M. Ottinger, University of Houston / Biology and Biochemistry; C. Geiselman, The Cullen Trust for Health Care

One Health provides a broad framework to conceptualize the close interactions and interdependence of human-wildlife-ecosystem health. Anthropogenic factors have introduced a range of stressors that impact all aspects of these dynamic interactions. These stressors arise from a variety of sources, including industry and urbanization pressures, land use, disease, and climate change. Wildlife conservation is fundamental to sustaining healthy ecosystems, which in turn interact with the health of humans and communities. The Exposome defines the health of an individual according to a point in time or as a cumulative reflection of lifetime exposures to environmental chemicals, microbes and other stressors through air, water, diet, living conditions, lifestyle and other sources. These conceptual frameworks reflect the power of convergent science related to the human-wildlife-ecosystem health nexus. We propose bridging the gaps that exist in each conceptual framework to produce a unified framework that accommodates both approaches.

07.04.08 Climate Change: Is It Real, a Hypothesis, a Hoax or Our Imagination?

R.G. Stahl, DuPont (retired)

The debate on whether climate change is real or not continues unabated across the globe. The debate is fueled by politicians, various on-line and social media platforms, among many others. Each of these has a view on climate change, one way or the other, that may or may not comport with the published scientific research that has been accumulating for decades. Our colleagues in the social science field tell us why people feel the way they do about climate change. It seems our personal experiences, information from close friends or family members, co-workers or peer pressure, and especially today our social media connections, are the drivers that cause us to “believe” something. These drivers appear to be as powerful, or more so, than what we learn from scientific research. And, yes, sometimes our religious beliefs have considerable influence on us. In this presentation, we will cover why these drivers persist when it comes to climate change, and the importance of scientific research to counter them.

07.05 Microplastic Monitoring and Risk Characterization for Management Strategies

07.05.01 California Microplastics Health Effects Workshop: Informing Management Strategies for the Aquatic Environment

L. Thornton Hampton, Southern California Coastal Water Research Project Authority / Toxicology; A.C. Mehinto, Southern California Coastal Water Research Project / Toxicology; S.L. Coffin, University of California, Riverside / Environmental Toxicology; E. Miller, San Francisco Estuary Institute; C.M. Rochman, University of California, Davis / Department of Ecology and Evolutionary Biology; S. Weisberg, Southern California Coastal Water Research Project

In 2018, the California Legislature adopted a pair of bills that require the State to begin building microplastics management strategies for both drinking water and California’s aquatic ecosystems. Senate Bill 1422 requires the California State Water Resources Control Board to develop plans for measuring microplastic particles in drinking water by 2021. Senate Bill 1263 requires the California Ocean Protection Council to adopt and implement a statewide strategy for lessening the ecological risks of microplastics to coastal marine ecosystems. To provide a scientific foundation for these legislative mandates, an international group of experts and environmental managers were convened for a Microplastics Health Effects Workshop in late 2020. The workshop addresses both human and environmental health effects, though we exclusively focus on the approach and outcomes for ambient waters here. Overall, the goal of this workshop was to identify the primary pathways by which microplastics affect biota, prioritize the microplastic characteristics that are of greatest biological concern, and identify critical thresholds at which those biological effects become pronounced. To achieve these goals, existing literature was collated to create a database focused on the toxicological effects of microplastics in aquatic organisms. Workshop participants then used the database and an accompanying RShiny web application to identify key drivers of toxicity, and a framework for the development of environmental thresholds was created. Data deemed fit for purpose were used to calculate thresholds for the ambient environment. Each threshold is directly associated with a specific management action. Finally, notable data limitations were documented throughout the workshop, and specific research needs for addressing critical data gaps and increasing confidence in these thresholds were provided. The outcomes from this workshop will be directly used by California environmental managers to provide context for microplastic monitoring programs and guide microplastic research initiatives moving forward.

07.05.02 A Proposed Framework for Microplastics Risk Assessment

K. Bucci, University of Toronto / Department of Ecology and Evolutionary Biology; C.M. Rochman, University of California, Davis / Department of Ecology and Evolutionary Biology

Microplastics (plastic < 5 mm) are unlike other environmental contaminants, because they are both a physical and a chemical stressor. Their global ubiquity and perceived harm has led governments around the world to express the need for a risk assessment on microplastics, or at least a framework for performing one. However, exercises aiming to conduct a risk assessment end up in conversations about whether to treat the contaminant as either a particle or a chemical, both of which fail to capture the reality of microplastics in the environment. Microplastic pollution is a complex mixture of particles of different shapes, sizes, and polymer types, and with a complex and dynamic suite of chemical additives and sorbed environmental chemicals. This complexity has made translating existing ecotoxicological studies into an assessment of environmental risk difficult. To effectively assess the risk that microplastics pose, a framework is required that captures the hazards associated with both their physical and chemical characteristics. Here, we propose a novel framework for evaluating the hazard of microplastics as a complex, multi-dimensional contaminant. We have identified five dimensions of microplastics that are relevant to the hazard of the particle. These are: size, shape, polymer type, additives from manufacturing, and sorbed environmental contaminants. Using the available literature, we have ranked the characteristics of these five dimensions from least to most harmful to inform the hazard. For instance, strategic effects testing has suggested that spherical, additive- and environmental contaminant- free microplastics are the least harmful formulation of microplastics. Using the rankings, we can calculate a hazard value associated with a particular microplastic particle, which informs how harmful that particle is to an organism based on its physical and chemical characteristics. For a given microplastics sample, we can then calculate an overall risk score, by combining the hazard of each particle in the sample with exposure data. Our framework will provide decision-makers with the information needed to prioritize regulation of the microplastics that are doing the most harm in the environment.

07.05.03 Interlaboratory Method Evaluation Study for Microplastics - Informing Methods to Monitor Drinking Water in the State of California

H. De Frond, University of Toronto / Ecology and Evolutionary Biology; L. Thornton Hampton, Southern California Coastal Water Research Project Authority / Toxicology; S. Kotar, K. Gesulga, C. Matuch, W. Lao, Southern California Coastal Water Research Project Authority; C.S. Wong, Southern California Coastal Water Research Project Authority / Chemistry; C.M. Rochman, University of California, Davis / Department of Ecology and Evolutionary Biology

California senate bill 1422 requires that the California State Water Resources Control Board develop standardized methods for quantifying and characterizing microplastics in drinking water. To inform standardized methods, we led an interlaboratory microplastics methods evaluation study starting in 2019, with 23 participating laboratories from six countries. The aim of this study was to evaluate several common methods (extraction via filtering/sieving, microscopy, FTIR spectroscopy, and Raman spectroscopy). We measured recovery, precision, cost, and time for each stage of analysis. Three spiked samples of clean water, and a laboratory blank were provided to each lab that followed a prescribed protocol to analyze the samples. Samples contained a known amount of microparticles within four size fractions (1-20 µm, 20-212 µm, 212-500 µm, >500 µm) and with four polymer types (PE, PS, PVC, and PET) of various shapes and colors. Thus far, 10 labs have provided results for all size fractions. Here, the mean particle recovery was 55±10% (standard error) and ranged from 10% to 115%. For labs that identified particles greater than 20 µm, mean particle recovery was 91 ±14% (standard error) and ranged from 13% to 189%. The average particle count reported within blank samples was 91 particles, with a range of 7 to 511 particles per blank sample across labs. For identification of microplastics, 89% and 91% of spiked plastic particles analyzed were correctly identified by polymer type using FTIR and Raman spectroscopy, respectively. The average time spent per sample was three hours for microscopy, 13 hours for FTIR, and 15 hours for Raman spectroscopy. The cost varied among labs, but the highest upfront cost was for a Raman spectrometer. The results demonstrate workable recovery for particles greater than 20 µm in size, with promise for increased recovery of smaller particles based on high precision. Results from this study are currently informing ongoing research and methods for extraction, quantification, chemical identification, and contamination control for monitoring microplastics in drinking water in the State of California.

07.05.04 Assessing Risks of Microplastics in Drinking Water for Humans: Insights from California's Expert Workshop

S.L. Coffin, University of California, Riverside / Environmental Toxicology; H. Bouwmeester, WUR; S.M. Brander, Oregon State University / Environmental and Molecular Toxicology; T. Gouin, TG Environmental Research; L. Hermabessiere, University of Toronto / Ecology and Evolutionnary Biology; E. Khan, California Office of Environmental Health Hazard Assessment; A. Koelmans, Wageningen University / Aquatic Ecology and Water Quality Management Group; M. Wagner, Norwegian University of Science and Technology / Department of Biology; S. Weisberg, Southern California Coastal Water Research Project; S. Wright, Kings College London

Recent findings of microplastics in drinking water prompted concern amongst scientists and policymakers due to the potential harm towards humans. In 2018, the California legislature passed Senate Bill 1422 requiring the California State Water Resources Control Board to (1) define microplastics, (2) develop a standardized method to monitor in drinking water and accredit laboratories, (3) monitor for microplastics in drinking water for four years, and (4) provide consumers with guidance regarding health impacts. Despite being studied since 1961, the human health effects of microplastics remain poorly understood, especially through the ingestion route of exposure. However, the exponential increase in the number of mammalian toxicity studies since 2019 allows for preliminary insights into potential human health effects. To assess the available evidence for human health hazards of microplastics in drinking water, we convened an inter-sector expert workshop, and conducted a systematic literature search. Studies were screened for quality criteria, including particle characterization, experimental design, and applicability for risk assessment prior to undergoing additional expert evaluation. *In vivo* and *in vitro* studies suggest microplastics may cause adverse effects in model mammalian organisms to various target organs through oxidative stress and inflammatory pathways, however significant uncertainties remain for both hazard identification and exposure assessment. Challenges in estimating risks from exposure to microplastics include a relative lack of studies on polymers and shapes other than polystyrene spheres, uncertainties with measured apical endpoints, and missing exposure information. Insights from the expert elicitation process, quality scoring tool, and key findings from the available toxicity studies will be presented.

07.05.05 Chicken and Egg on a Single Plate? On the Road of Standard Development and Interlaboratory Study on the Quantification of Microplastics in Water

D. Mehn, European Commission-Joint Research Centre; A. Held, European Commission - Joint Research Centre / Institute for Reference Materials and Measurements Standards for Innovation and Sustainable Development; S. Belz, European Commission - Joint Research Centre; H. Emteborg, European Commission Joint Research Centre Institute for Reference Materials and Measurements IRMM; J. Seghers, European Commission Joint Research Centre; E.A. Stefaniak, P. Robouch, J. Stroka, R. La Spina, C. Cella, F. Fumagalli, European Commission Joint Research Centre; D. Gilliland, European Commission, Joint Research Centre / Directorate F Health, Consumers and Reference Materials; B. Sokull-Kluettgen, European. Commission - Joint Research Centre / Nanobiosciences

In the presented work, we tried to get closer to the solution of the classical chicken and egg problem of how to develop harmonised methods without existing standards and how to develop standards without validated methods – this time for the quantification of microplastics in water. Due to the diversity of techniques and procedures applied to determine microplastics in various matrices and the lack or scarcity of quality assurance tools, such as validated methods and reference materials, a comparison between the performance of methods published in scientific literature and harmonisation based solely on literature data is not feasible. Two laboratories of the Joint Research Centre of the European Commission embarked together in the development of a microplastic reference material and shortly after launched an inter-laboratory comparison study to support the reference material development. The distributed samples consisted of polyethylene terephthalate particles in a salt cake and a bottle of water. Participant laboratories received a protocol for the reconstitution of the particles and generation of a "drinking water" model sample, but were free to use their usual analytical method and protocol and to report mass or number concentration of larger than 30 µm polyethylene terephthalate (or plastic) particles depending on the nature of their selected technique. At the end of the exercise, both the indicative mass and number concentration were shared with the participants. The study aimed also the deeper understanding of the existence and attributes of methods applied by the scientific community for analysis of microplastics in water. Thus, participant laboratories were asked to fill in a survey that contained numerous questions regarding the details of their procedures. Thanks to their efforts as well, the information collected from 96 datasets provides an excellent overview of the current status of microplastics quantification procedures in water. Preparation process of the test material was published in a peer-reviewed journal; analysis of the data was shared with participants in the form of a report and discussed also at workshops. Even if the concentration values found by the different laboratories were widely scattered around the centres of the indicative ranges of expected values in case of all applied methods, the results and discussion with participants suggest that the chosen sample preparation strategy and particle concentration is promising for the preparation of a future microplastic reference material.

07.05.06 Addressing the Multidimensionality of Microplastic in Order to Consistently Characterise Risk

M. Kooi, A. Koelmans, Wageningen University / Aquatic Ecology and Water Quality Management Group

Assessing the risks of microplastics for the environment and human health is generally considered extremely difficult. In 2015 – 2021, the Dutch National programme 'Technologies for the Risk assessment of Microplastic' was performed in order to support water boards and environmental & human health institutions in The Netherlands with respect to their environmental management tasks. A framework has been developed that combined QA/QC criteria for exposure and effect assessment, rescaling methods and (probabilistic) modelling approaches to provide a consistent risk characterisation while fully accounting for the multidimensionality of environmental and/or dietary microplastic. Rescaling methods were developed that correct for the differences in size ranges targeted in studies reporting microplastic concentrations. This includes methods to interchange between threshold effect concentrations expressed using dose descriptors such as number, volume, area, specific surface area, shape and mass concentrations using probability density functions (PDFs). PDFs based on the characteristics measured for > 60,000 individual particles in situ, accurately reflect the characteristics of microplastic particles in the environment or in food, while also representing the dose descriptors as they are known from (eco)toxicologically relevant particle toxicity mechanisms. PDFs are used to correct for the incompatibility of data as applied e.g. in species sensitivity distributions (SSDs), caused by differences in the microplastic types used in effect studies and those in nature. It is illustrated how threshold effect concentrations can be derived and compared with aligned exposure data to consistently characterise risks. Altogether, the toolset allows us to accommodate the diversity of microplastic, to address it in a common language, and to assess its risks as one environmental material.

07.05.07 Sources, Pathways, Prevention, and Mitigation of Fiber Pollution in Urban Runoff

D. Lin, K. Moran, E. Miller, M. Mendez, S. Moore, T. Hale, R. Sutton, San Francisco Estuary Institute

Fibers are among the most widely reported microplastic pollutants reported globally, and yet the major sources and pathways are not well understood, which limits development of management options to mitigate or capture fiber pollution. Recent investigations in San Francisco Bay identified urban runoff as a major pathway for microplastics and fibers, with more fibers estimated to enter receiving waters from urban stormwater runoff than from wastewater annually. This indicated that key questions about dominant sources of fibers need to be investigated to inform local, national, and global microplastic management strategies. Fibers and associated chemical additives pose toxicological risk to aquatic life. We developed conceptual models to synthesize and integrate our current understanding of fiber sources and pathways to urban runoff and to inform future research and recommendations for managing fiber pollution. Here, we present a state-of-the-science review of the potential sources of fibers and occurrence and fate of fibers in urban environments, focusing on how these pollutants may enter urban runoff. We also present a broad framework for developing control strategies, focusing on fibers from textiles. There are a wide array of management approaches available, including re-designing how textiles and finished garments are manufactured, reducing fiber fragments released, using fiber capture technologies in dryer appliances and building exhaust systems, and removing fibers in urban runoff. These control strategies highlight the wide range of opportunities to mitigate fiber pollution, and the necessary collaborations among industry, government, and consumers.

07.05.08 An Ecological Risk Assessment for Microplastics in the San Francisco Bay Using the Bayesian Network Relative Risk Model

E. Sharpe, S. Elmstrom, E. Whitney, Western Washington University / Institute of Environmental Toxicology and Chemistry; S.L. Harper, Oregon State University / EMT; W.G. Landis, Western Washington University / Institute of Environmental Toxicology and Chemistry

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 microplastics for 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 risk for Chinook salmon, Olympia oysters, Northern anchovy, Pacific herring, and human health/wellbeing. 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. We have chosen to break our conceptual models into two different pathways, (1) tire wear particles and (2) other microplastics, based on likely differences in sources, transport, and toxicity. Our study site is broken into four risk regions based on watershed boundaries and land use patterns. The BN-RMM is parameterized for each risk region using microplastic abundance 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 of the four risk regions and spatial gradients of risk were determined. This study is funded by the National Science Foundation Growing Convergence Research Grant (1935018) program.

07.05.09 Microplastic Pollution in California: Using Scientific Guidance to Inform a Precautionary Framework that Assesses and Addresses Risks to Marine Ecosystems

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In 2020, a group of experts was convened by the California Ocean Science Trust to assess the state of the science in the field of marine microplastics and construct a framework that would accurately assess potential risks to marine ecosystems in California. The group of toxicologists, ecologists, chemists and risk assessors drawn from academic, government, and private sectors were also charged with identifying actions to be taken in light of threats to resident marine species and habitats. Following several months of work and consideration, key recommendations arising from this process were that California should use a precautionary approach to assess and manage microplastic pollution risk, because there are no feasible cleanup options for this class and size of persistent and widely dispersed pollutants in marine water, and because projections point to a predicted increase in environmental concentrations. The current body of knowledge in the greater field strongly suggests that microplastic exposure at sufficient dose can lead to adverse effects in organisms and humans. High priority components of the framework and risk prioritization tool developed from the group include a) consideration of certain particle morphologies and polymer types (e.g., fibers, tire wear particles), b) focus on specific fate and transport pathways such as stormwater runoff and aerial deposition, c) characterization of major sources in the state (e.g. tire and road wear, laundry / textiles), and d) emphasizing priority endpoints that constitute taxonomic groups of highest concern, both ecologically and in terms of importance as fisheries (e.g. mollusks, crustaceans, fish). This presentation will describe the risk prioritization tool that was developed and recommended source reduction strategies and data gaps that were highlighted by the working group. Future steps concerning California's plan for reducing plastic pollution and supporting data collection efforts to increase the existing knowledge base over the next five years will also be described. The broad recommendations issued in this report represent the first such effort in the United States, and thus progress made since the report was published, in both legislative and research arenas will also be highlighted.

07.05.11 The Role of Spatial Models in Microplastic Research Relevant for Policy Action - an Example From a River Basin in Germany

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Microplastic (MP) monitoring programs in terrestrial and freshwater environments are planned by many countries. These efforts are important, since policy priorities should be based on risk assessment, which includes the analysis of exposure concentrations. However, as an isolated approach, monitoring entails some caveats: even an extensive sampling scheme will only be able to produce limited, spotty results. Measurement uncertainties and analysis labour increase with decreasing microplastic concentrations that are expected especially in undisturbed regions sampled to identify background threshold values. Moreover, at the current state of knowledge, the relevance of the numerous microplastic fluxes into the environment and their spatial distribution is still greatly unknown. Modeling approaches address these constraints and therefore play a crucial role in policy-informing research programs that are currently being developed at different administrative levels. In the Weser catchment, a major tributary to the North Sea, we developed an interdisciplinary modeling framework to estimate MP emissions into agricultural land, freshwater ecosystems and further to the estuary and subsequent transport to the adjacent sea. Using data from field campaigns, literature, statistics, hydrologic monitoring, as well as hydro- and morphodynamic parameters, three models of disparate origin were linked to describe MP emissions, pathways and fate across ecosystem boundaries. We present a first preliminary assessment of microplastic quantities emitted by the application of sewage sludge, compost, and plastic mulch film in agriculture, as well as point and diffuse emissions into surface waters. Further, we describe the transport behaviour within the Weser estuary and coastal zone of the North Sea. Such area-covering results are required to understand MP abundance, behaviour, and transport mechanisms across ecosystems at scales relevant for policy-making. This framework can be transferred to other regions and refined continuously with improved parameters and assumptions. We conclude that models should precede and accompany monitoring programs to support meaningful data generation with limited sampling and analysis capacities. Moreover, models provide a systems approach to the emerging research field on plastics in the environment needed to inform effective mitigation strategies.

07.05.12 Impact of Microplastics on Marine Ecosystems and Human Health in South Kalimantan, Borneo, Indonesia

C. Lee, University of Newcastle (Australia) / School of Environmental and Life Sciences; A. Budiman, Universitas Lambung Mangkurat / International Division

The focus of this paper is a short review of plastics and microplastics pollution in Indonesia. Additionally, a specific research study on the impact of microplastics on the marine ecosystems and human health in South Kalimantan, Indonesia will be discussed. Among all the 10 ASEAN (Association of Southeast Asian Nations) countries, an extensive 2019 review of research on marine plastics in Southeast Asia showed that Indonesia stands out as the country that "attempts to understand more aspects of the marine plastics problem than all other Southeast Asian countries – it is the only country with the assistance of the World Bank tried to identify hotspots and accumulation zones of marine plastics." In terms of plastic waste, Indonesia ranks number 2 out of the top 120 coastal communities that mismanaged plastic waste in 2010. As much as 0.48 to 1.29 million metric tonnes per year of plastic marine debris leaked into the marine environment. This is a portion of the 3.22 million tonnes of mismanaged waste in 2010. Plastic composition of solid waste was estimated at 11% to 15%. These estimates are not surprising given the Indonesian population of 270 million living in an extensive land mass with 17,000 islands encompassing over major waterways and oceans. Therefore, it is imperative that an urgent call to address research emphasis on plastic and associated microplastics accumulation and impacts in the marine ecosystems as well as understanding the uncertainties of such microplastic pollutants on human health is needed. Indonesia has the most extensive research in ASEAN on marine plastics: an overview is provided focusing on beach macro-litter monitoring, monitoring in the environment, co-pollutants, microplastic ingestion by microorganisms and community empowerment. In South Kalimantan, Borneo, the University of Newcastle (Australia) has established a long-term research collaboration with the Universitas Lambung Mangkurat on understanding the conservation of the mangrove habitat that is home to an endemic endangered species - proboscis monkey (*Nasalis lavartus*). Focusing on the sensitive Barito river mangrove ecosystem, we plan to conduct a preliminary human health risk assessment of plastics in the local village community and Banjarmasin city. The aim is to evaluate the impacts of plastics and microplastics posed to the health of villagers living in the village community as compared with the urban residents living in the city. A survey study will be conducted to evaluate the plastic exposure from food intake, inhalation, and dermal exposure. A survey of human dietary intake will also be conducted. Samples will be collected from the river, drinking water, specific aquatic biota, food items and air for microplastics analyses. The results will hopefully be used to drive home a powerful message to the community to reduce plastic usage and eliminate illegal dumping into the rivers.

07.05.13 Sources, Pathways, Prevention, and Mitigation of Cigarette Butt Litter and Cellulose Acetate Fiber Pollution in Urban Runoff

E. Miller, D. Lin, M. Mendez, S. Moore, T. Hale, K. Moran, R. Sutton, San Francisco Estuary Institute

Recent investigations in San Francisco Bay identified urban runoff as a major pathway for microplastics to enter receiving waters, with more fibers estimated to enter receiving waters from stormwater than from wastewater annually. Cellulose acetate fibers in urban runoff are frequently linked to cigarette butts, which are one of the most commonly littered items. Cigarette butts and associated contaminants, including cellulose acetate fibers, pose toxicological risk to aquatic life. We developed conceptual models to synthesize and integrate our current understanding of cellulose acetate microplastic sources and pathways to urban runoff and to inform future research and management recommendations for managing cigarette butt and cellulose acetate fiber pollution. Here, we present a state-of-the-science review of the occurrence and fate of cigarette butts and cellulose acetate fibers in urban environments, focusing on how these pollutants may enter urban runoff. We also present potential control strategies for reducing butt litter and microplastic release. There are many available approaches to management, including re-designing cigarettes, removing plastic ingredients, education to decrease smoking frequency and butt littering, and downstream approaches to collect trash and microplastics on land and in water. These control strategies highlight the wide range of opportunities to control cigarette butt and cellulose acetate fiber pollution, and the necessary collaborations among industry, government, and consumers.

07.05.14 Sources, Pathways, Prevention, and Mitigation of Single-Use Plastic Foodware (SUPF) and Related Microplastics in Urban Runoff

M.A. Mendez, S. Moore, D. Lin, K. Moran, T. Hale, R. Sutton, San Francisco Estuary Institute

The use of plastics globally continues to drastically increase, especially for items intended for short-term use and disposal. Single-use plastic foodware (SUPF), broadly defined as disposable plastic items designed for single use to serve, package, transport, and/or consume prepared food and/or beverages, are a significant portion of plastics used and, ultimately, polluted into the environment as SUPF are among the most commonly littered items. However, the major sources and pathways as well as the breakdown of SUPF into secondary microplastics are not well established. Recent investigations in the San Francisco Bay have identified urban runoff as a major pathway for microplastics and fibers to enter receiving waters. We developed conceptual models to synthesize and integrate our current understanding of SUPF sources and pathways to urban runoff and to inform future research and management recommendations for managing SUPF pollution. Here, we present a state-of-the-science review of the potential sources of SUPF, occurrence and fate of SUPF in urban environments, focusing on how these pollutants may enter urban runoff. We also present a broad framework to identify control strategies for reduction of SUPF litter and microplastic release. This includes a variety of management actions such as product reformulation to develop more sustainable sources unlinked from fossil fuels, education to reduce littering, and improved collection, separation, and recovery of trash. These control strategies highlight the vast range of opportunities to mitigate SUPF pollution, and the necessary collaborations among industry, government, and consumer actors. [SM1]Length check: 1792 characters (with spaces). Limit 2500.

07.06 Pathways and Pitfalls: Achieving Science-Informed Solutions to Environmental Risk Challenges

07.06.01 Community Engagement - Balancing Community Concerns and Risk Perception

L. Wirtis, R. Wells-Albers, Oregon Department of Environmental Quality

The Oregon Department of Environmental Quality is the state agency responsible for restoring, maintaining and enhancing the quality of Oregon's air, land and water. The Pacific Northwest corner of the United States in general and Portland, Oregon in particular are places where communities are deeply invested and involved in environmental decisions that impact their lives. While cleaning up legacy environmental contamination isn't new, the cleanup process is not widely understood by the public. Agencies like DEQ are looking for ways to engage with the community and to share information beyond the typical, required public notice. This was the case at the Willamette Cove Upland site, which borders the Portland Harbor Superfund site along the Willamette River in the heart of Portland. Previous industrial activities resulted in significant soil contamination, posing a risk to human health and the environment. DEQ proposed to clean up the site by removing hot spots, consolidating some contaminated soil into a containment area, and covering the site with at least one foot of clean soil. DEQ worked with a local advocacy group on a program called "Train the Trainers" in which DEQ participated in education sessions to explain the science of the cleanup to community members who could then bring more people into the public comment process for the final cleanup plan. Train the Trainers included a two-day education session with the "trainers" as well as regular meetings. The education sessions included tutorials and discussions on cleanup concepts such as what is risk, how DEQ balances different factors in evaluating cleanups, and providing comments. Early on, the community's preference for complete removal of all contamination was clear. The guiding principle for successful community engagement is often reaching a solution meets both the regulator and communities' requirements and expectations. Willamette Cove provided many lessons, including how to demonstrate that community participation and time are valued, supporting meaningful engagement, creating a continuous flow of information to and from the community and agency partners, and demonstrating that people have impacted your final decision. In the end, DEQ found that public engagement wasn't about everyone agreeing, but about building trust and staying in the conversation.

07.06.02 Scientists' Weighting of Risks and Uncertainties Can Lead to Opposing Policy-Advice - a Case-Study of a Waste-Water Treatment Controversy

M. Vazquez, E. Eronen, University of British Columbia; G. Oberg, University of British Columbia / IRES

Sewage-related policy decisions rely heavily on scientific and technical advice but experts do at times disagree on suitable solutions. One example is the Capital Regional District (CRD), British Columbia, Canada, where scientists have publicly positioned themselves either against (anti-treatment) or in favour (pro-treatment) of building a wastewater treatment (WWT) facility. The aim of the present study is to analyse the relative weight scientists from the two sides give to the risks and uncertainties they associate with the problem. Interviews with scientists from each side of the controversy suggest that the two sides give different weight to different types of uncertainties, and also differ in their judgements regarding which measures are most appropriate to reduce those uncertainties. This is, for example, seen in how they interpret the absence of warning signs from the monitoring program. The anti-treatment scientists saw this as sufficient to conclude that discharging the sewage untreated at that location is a safe practice. In contrast, pro-treatment scientists argued that the absence of evidence of harm in the monitoring program was insufficient to conclude that the practice was safe. They maintained that this is because the programme did not include important determinants of harm (e.g. biomagnification or organic micropollutants, such as PCBs). The anti-treatment scientists advised that building a WWT plant was questionable, in part because it is uncertain if such treatment would be effective in relation to substances of concern. Contrarily, the pro-treatment scientists saw the potential presence of harmful substances as a sufficient reason to build a WWT facility. When it comes to uncertainties, they saw treatment as a way to reduce the potential harm posed by such substances. In contrast, the anti-treatment scientists emphasized the need for more research to reduce uncertainties surrounding sources, fates and effects of emerging contaminants, and the relative impact of different measures. Hence, the two sides exemplify how different weighting of risks and uncertainties can lead to opposing policy-advice. Our study suggests that research into the relative weight given to different types of uncertainties has the potential to support the construction of frameworks that can facilitate transparent communication of different perspectives in science-advice and thus, by extension, support more democratic policy decisions.

07.06.04 Application of Causal Analyses to Evaluating Ecological Changes in a River and Estuarine System

C. Menzie, A. Morrison, A. Deines, Exponent / Ecological & Biological Sciences

This presentation describes the use of formal causal analyses incorporating weight-of-evidence criteria for unravelling the causes of an array of environmental changes. Using a water rights case for illustration, we describe a series of tiered causal analyses to investigate ecological impairments and alterations involving algal blooms, flooded wetlands, freshwater mussels, and fish populations. While the case focused on upstream water consumption, our analyses also examined the role of associated stressors including nutrients, salinity, intrusions of predators, flow modifications, and long-term desiccation. The presentation will cover advantages of using formal causal analysis, including providing a framework for organizing information and supporting expert opinion, assessing the adequacy of evidence, and avoiding cognitive bias. The presentation will illustrate how SETAC sciences can be used in an interdisciplinary way within this methodology.

07.06.05 The Value of Increased Spatial Resolution of Pesticide Usage Data for Assessing Risk to Endangered Species

E.L. Murphy, Arizona State University / School of Life Sciences; S. Eikenberry, G. Iacona, Arizona State University; G. Watson, Bayer CropScience; L. Gerber, Arizona State University

Under the U.S. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), EPA is responsible for registering and reviewing all pesticides. EPA's obligations under Section 7 of the Endangered Species Act (ESA) require the registration and review process to consider potential impacts of pesticides to listed endangered species and critical habitats, and for the Services –U.S. Fish and Wildlife Service (USFWS) and National Marine Fisheries Service (NMFS)—to complete a formal Section 7 consultation if any species is deemed likely to be adversely affected. The ESA consultation process for pesticide registration and review is time intensive and lacks transparency and confidence among stakeholders. To examine the efficiency and confidence of the consultation process, we evaluated the relative importance of spatially explicit, high resolution pesticide usage data for the carbaryl risk assessment process. We found that spatially explicit, township resolution usage data (roughly 36 square miles) excluded roughly one third of terrestrial plants (55/168) endemic to California and half of their critical habitats (27/53) from requiring a full Section 7 consultation. In contrast, EPA only exclude 4% of terrestrial plants from requiring formal Section 7 consultation in their final biological opinion for carbaryl. This suggests high-resolution usage data could markedly increase pesticide review efficiency, save the US Fish and Wildlife Service years of man hours and millions of dollars, and ultimately decrease the amount of time pesticides remain on the market without a formal biological evaluation. Collaboration and communication with stakeholder groups could present further opportunities for increasing efficiency of and confidence in this process.

07.07 Regulatory Affairs and Emerging Concerns for Metals in the Environment

07.07.01 U.S. Environmental Protection Agency's Plans to Update Aquatic Life Ambient Water Quality Criteria (AWQC) for Metals

C. Bergeron, K. Gallagher, J.R. Beaman, L.A. Cruz-Rodríguez, M. Elias, U.S. Environmental Protection Agency / Office of Water

Aquatic Life Ambient Water Quality Criteria (AWQC) for toxic chemicals developed by the U.S. Environmental Protection Agency (EPA) are national recommendations of the highest concentration of specific pollutants or parameters in water that are not expected to pose a significant risk to the majority of species in a given environment. States, territories, and tribes may use the recommended criteria in developing their own standards. To date, EPA has developed AWQC for 10 metals, however with the exception of aluminum, cadmium, and copper, the remaining criteria were developed in the 1980s and 1990s and do not reflect new toxicity studies or approaches for considering how water chemistry parameters (*e.g*., pH, dissolved organic carbon, and hardness) can affect metal bioavailability and subsequent toxicity to aquatic species. In 2017, EPA signed a Cooperative Research and Development Agreement (CRADA) with eight metals associations to leverage the knowledge and resources of scientists inside and outside of the agency to better protect aquatic life from toxic metal exposure. The goal of the CRADA project is to develop a simplified, overarching modeling framework to predict the bioavailability of metals considering a common model parameter set, modeling approach and platform to apply towards updating AWQC for metals. EPA is using a two-phased approach to address the five-year CRADA. In Phase I, EPA worked with external technical experts from the metals associations to develop a proposed modeling approach to predict the bioavailability and toxicity of metals under the range of water chemistry conditions found in aquatic environments common in freshwaters of the United States. In Phase II, EPA is working with the metals associations to develop bioavailability models for individual metals using the overarching modeling approach. After 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 with criteria that reflect the latest science and are easier to implement than previous approaches using metals bioavailability modeling for criteria development. This presentation will summarize the current activities under Phase I and discuss the path forward for Phase II and criteria development.

07.07.02 Estimating Biotic Ligand Model Chemistry Input from Conductivity

K.E. Croteau, R.C. Santore, Windward Environmental, LLC; A. Kennedy, SUNY-Binghamton

The Biotic Ligand Model (BLM) has been widely used to explain and predict how water chemistry affects metal toxicity on aquatic organisms. The full list of chemistry parameters required for the BLM includes pH, dissolved organic carbon (DOC), major cations (Ca, Mg, Na, and K), major anions (SO4 and Cl) and alkalinity. In monitoring datasets that were not specifically designed to obtain complete chemistry for the BLM, one or more of these ions may be missing. For the past few years, the BLM software has had the ability to estimate the full chemistry inputs from relatively simple chemistry inputs. This approach uses hardness and geochemical ion ratios to estimate complete chemistry. The hardness-based simple chemistry estimation method has greatly expanded the number of waters where the BLM can be applied. The aim of this work was to further expand the ability to use the BLM with monitoring data by developing a methodology for converting measures of specific conductivity to the individual ions required for the full BLM chemistry inputs. This simple chemistry approach uses conductivity instead of hardness in a similar estimation approach. The methodology is based on previous work by others whereby conductivity can be estimated from the ionic composition. The relative proportions of the major cations (i.e., Ca, Mg, Na, and K) and anions (i.e., SO4 and Cl) are set with median molar ratios that have been determined for different geographic regions (calcium by each of the other 3 cations, and sulfate by chloride). Alkalinity and acidity species (i.e., CO3, HCO3, OH, and H) are calculated from pH and atmospheric carbon dioxide, assuming the water is in equilibrium with the atmosphere. Initial tests with this methodology have shown promising results, on par with the hardness-based simple chemistry estimation methods that are already in regular use with the BLM software. This presentation will take a closer look at the uncertainties and errors that can arise when this method is used, including how the results differ when the BLM is used to calculate water quality standards.

07.07.03 Expanding the Water Quality Boundaries for Application of the Nickel and Zinc Biotic Ligand Models

R.C. Santore, K.E. Croteau, Windward Environmental, LLC; A.C. Ryan, International Zinc Association; E.T. Middleton, E.R. Garman, C.E. Schlekat, NiPERA Inc; E.J. Van Genderen, International Zinc Association; J.M. Besser, U.S. Geological Survey / WFRC; C.D. Ivey, U.S. Geological Survey / PWRC - Beltsville Lab; D. Cleveland, J.A. Steevens, U.S. Geological Survey / Columbia Environmental Research Center; D.J. Soucek, A. Dickinson, University of Illinois, Urbana-Champaign / Illinois Natural History Survey

Updates to water quality guidelines in jurisdictions around the world have increasingly considered bioavailability modeling to improve the accuracy of the regulation to local conditions. Although bioavailability models are developed and evaluated using datasets that examine the effects of toxicity modifying factors (TMFs) on toxicity, the guideline derivation rarely considers how TMFs affect the metal that is covered by the guideline. Historically, linear regressions have been used to consider hardness effects on toxicity, but these equations were typically limited to the range of hardness values in datasets used to calculate the hardness slope. With the more recent use of bioavailability models such as the Biotic Ligand Model (BLM) and multiple linear regression (MLR) model boundaries need to be defined for all the TMFs considered by the model. Although most site waters fall within the ranges of conditions covered by calibration and validation datasets s, there are some cases where these boundaries are not sufficient to cover all conditions that may be encountered in site waters where the guideline will be applied. It is useful, therefore, to consider targeted testing to expand the range of conditions where model performance can be tested and validated. As part of a study to investigate metal toxicity within natural waters exhibiting extreme pH and DOC conditions, Ni and Zn toxicity tests were conducted with the cladoceran, *Ceriodaphnia dubia*, and the mayfly, *Neocloeon triangulifer*. Test waters included two water types that are typically under-represented in toxicity tests. These include the high pH, high hardness, and high alkalinity conditions in the 'corn belt' of central Illinois, U.S., and the low pH, soft water and high DOC characteristic of boreal forest of northern Minnesota U.S.). Overall, BLM and MLR models successfully modeled variation in toxicity of both metals to both species across a wide range of water chemistry. Successful predictions of toxicity to a standard and a novel test organism in diverse water chemistries demonstrates the reliability of these models as for basis for water quality criteria to protect aquatic organisms.

07.07.04 Application of a Copper Site-Specific Objective Study in Marina Del Rey Harbor

A.N. Parks, Exponent Inc. / Toxicology; D.J. Greenstein, Southern California Coastal Water Research Project / Toxicology; K. Schiff, Southern California Coastal Water Res.

Copper is a contaminant of concern in Marina del Rey Harbor (MdRH) as monitoring and special studies in the Harbor have shown that dissolved copper concentrations frequently exceed the chronic (4-day average) criterion of 3.1 micrograms per liter (µg/L). The primary copper source in MdRH is copper-based anti-fouling paint used on boat hulls. The U.S. Environmental Protection Agency (USEPA)-approved Water Effect Ratio (WER) method was used to develop a copper site-specific objective (SSO) for MdRH, which considers the potential impacts of site-specific conditions on the bioavailability of copper. This WER study was developed in collaboration with a Technical Advisory Committee (TAC) following the USEPA guidance as well as methods used in two previous WER studies in San Francisco Bay and San Diego Bay. The study included six sampling events and was designed to capture a range of representative water quality conditions that exist in the Harbor, including factors such as seasonality, stormwater discharge, and hydrology. Two WER calculation methods were used: the standard 48-hour mussel embryo development toxicity test, and the Biotic Ligand Model (BLM). The toxicity-based (measured) and BLM-based (predicted) EC50 values for site water and reference water were used to calculate measured sample WERs (sWERs) and predicted sWERs. The measured sWERs ranged from 0.925-2.04, and the predicted sWERs ranged from 1.07-23.9. The dry weather predicted sWERs (1.07-1.65) were generally higher, but in the same range as the measured sWERs (0.925-1.44). The geometric mean of all sWERs resulted in a final WER (fWER) of 1.40 and 2.16 for the toxicity test and BLM methods, respectively. These fWERs are similar to those from San Diego Bay (1.33 and 1.67), and San Francisco Bay (2.40 and 2.77). Although both WER calculation methods suggest the site-specific conditions in MdRH are more protective than the reference water conditions, copper concentrations still exceed the proposed site-specific criterion. In addition to providing an SSO, other potential implementation actions such as following mandated hull cleaning best management practices, alternative hull paints, boat lifts, in-water dry docking, and improved public awareness are necessary to reduce the ecological risk.

07.07.05 Revising the Environmental Quality Standard for Nickel Under the European Water Framework Directive: A Focus Upon the Science or the Implementation?

A. Peters, G. Merrington, I. Wilson, WCA Environment Limited; E.T. Middleton, C.E. Schlekat, E.R. Garman, NiPERA, Inc.

Under the European Water Framework Directive (WFD) existing environmental quality standards (EQS), such as nickel, can be revised during a regular five-year review cycle. The key reasons for revision tend to focus upon new scientific data comes and/ or if there is a change in the extent and magnitude of potential EU-wide aquatic risks associated with the substance. The EQS for nickel was originally set at a concentration of 20 µg/L dissolved nickel, but was revised to take account of new information on its toxicity to aquatic organisms and to take account of bioavailability. The current nickel EQS under the WFD has been in place since 2013, is 4 µg/L and is set as a 'bioavailable nickel' concentration. This means that to assess compliance in Europe's freshwaters each country must take account of the bioavailability of nickel at each compliance assessment site. This requires concurrent measures of pH, dissolved organic carbon (DOC) and calcium, on each sampling occasion and the use a simplified bioavailability calculation tool, based on the nickel biotic ligand model. It has long been established that one of the practical barriers to the regulatory application of bioavailability approaches is the absence of measures of DOC. The European Commission is considering revising the nickel EQS, but has the potential for EU-wide risks changed in the last 5 years? Efforts have been made since the implementation of the EQS for nickel to simplify the compliance assessment for the regulatory authorities that assess compliance against the EQS for nickel. This has led to the development, and subsequent refinement, of the simplified bioavailability calculation tool "bio-met" (www.bio-met.net), and the development of guidance on the implementation of bioavailability based EQS for metals (although this has not yet been published). A tiered approach towards compliance assessment is a key aspect of the recommended implementation approach, which starts with a screening tier using dissolved nickel measurements, and identifies those sites where the additional supporting parameters for bioavailability assessment are required. When bioavailability-based methods are applied correctly to the nickel EQS compliance is extremely high. Although the current EQS for nickel has been in place for eight years there remain numerous challenges associated with properly implementing the current EQS that result in uncertainty surrounding the true compliance situation for the nickel EQS in Europe.

07.07.06 Updating the EQS for Nickel in Europe

D. Leverett, A. Peters, G. Merrington, WCA Environment Limited; E.T. Middleton, C.E. Schlekat, E.R. Garman, NiPERA, Inc.

The Environmental Quality Standard (EQS) for nickel in Europe under the Water Framework Directive has used a bioavailability-based approach since it was first established in 2013, and the same EQS applies throughout Europe because nickel was identified as a Priority Substance. The information that was used for the derivation of the EQS was compiled in 2010 and since that time there have been several advances in the science upon which it was based, and whilst none of these individual developments call into question the basis for setting the EQS for nickel they do offer the potential to improve upon it. The scientific developments that have been made broadly cover two areas, these are the range of water chemistry conditions that the bioavailability models can be applied over, and the ecotoxicity dataset to which the models are applied to derive a local EQS value. Testing of the bioavailability models has resulted in developments allowing them to be applied to higher pH conditions and in softer waters than was possible previously. A larger proportion of European surface waters can be included in bioavailability-based compliance assessments, predominantly due to the increased pH range of the updated models. The extended range of water chemistry conditions means that more sensitive water types can be included in the assessment. Additional toxicity test data means that there are an additional 15 species that were not previously represented in the ecotoxicity database, bringing the total number of species included to 47. There are also a much larger number of species for which multiple toxicity tests are available covering a range of different water chemistry conditions. This means that it is now possible to evaluate which of the bioavailability models is most appropriate for the normalisation of toxicity data for virtually all groups of taxa, leaving few for which bioavailability corrections must be performed with multiple models to select the most conservative result. There are also additional mesocosm and field studies available which collectively suggest that the local EQS values derived by the updated approach are sufficiently protective of long-term adverse effects on the algal and invertebrate communities that the studies considered. An assessment of the sensitivity of European surface waters indicates that an updated annual average EQS for nickel would be lower than the existing EQS, although this is principally due to the inclusion of more sensitive waters.

07.07.07 Developing a Bioavailability-Based Environmental Quality Standard for Nickel in China

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Recent scientific developments on the behaviour, fate and ecotoxicology of nickel have resulted in the development of both semi-mechanistic and empirical bioavailability models for nickel that can be used for regulatory purposes. These models have been applied in the derivation and application of the annual average environmental quality standard (AA EQS) for nickel under the European Union's Water Framework Directive for some years, and similar approaches are also under consideration for similar regulatory applications in both North America, Australia, and China. Regulatory authorities in several regions have needed to demonstrate the applicability of the bioavailability normalisation approach either for their local surface waters, local ecotoxicity test species, or both. The consideration of bioavailability-based approaches for understanding and regulating nickel emissions in China also requires information to demonstrate the validity of these approaches for the local water chemistry conditions, and also may need to be demonstrated for specific Chinese species. Testing has already been performed using standard test species (*Pseudokirchneriella subcapitata*, *Daphnia magna*, and *Brachydanio rerio*) in a variety of field collected Chinese waters exhibiting a range of different water chemistry conditions. Both semi-mechanistic and empirical existing bioavailability models have been able to provide acceptable predictions of the differences in sensitivity observed between the different water chemistry conditions in these natural waters. Regulatory guidance for the derivation of environmental quality standards in China allows for the use of a species sensitivity distribution if sufficient appropriate ecotoxicity data are available. However, performing a bioavailability normalisation for an entire ecotoxicity dataset requires that there is sufficient information on the water chemistry conditions in the tests that are included to enable to bioavailability to be properly taken into account. This can be an important factor in determining the amount of suitable high-quality data that is available for inclusion in the standard derivation. Validatory testing may also be required for key Chinese species in order to ensure that important and potentially sensitive local species will be adequately protected by any water quality standard that is derived for nickel, and that any bioavailability-based approaches are also applicable to these key species.

07.07.08 Environmental Risk of Nickel in Aquatic Arctic Ecosystems

P.T. Gauthier, T. Blewett, University of Alberta / Biological Sciences; E.R. Garman, C.E. Schlekat, E.T. Middleton, NiPERA, Inc.; A. Cremazy, University of New Brunswick, Saint John / Biological Sciences

The Arctic faces many environmental challenges, including the continued exploitation of its mineral resources such as nickel (Ni). The responsible development of Ni mining in the Arctic requires establishing a risk assessment framework that accounts for the specificities of this unique region. We set out to conduct preliminary assessments of Ni exposure and effects in aquatic Arctic ecosystems. Our analysis of Ni source and transport processes in the Arctic suggests that fresh, estuarine, coastal, and marine waters are potential Ni-receiving environments, with both pelagic and benthic communities being at risk of exposure. Reported environmental concentrations of Ni show that most contaminated sites are located in freshwater environments, but there is a clear lack of data for coastal and estuarine environments near Ni mining operations. Nickel bioavailability in Arctic freshwaters seems to be mainly driven by dissolved organic carbon (DOC) concentrations with bioavailability being the highest in the High Arctic, where DOC levels are the lowest. However, this assessment is based on bioavailability models developed from temperate species ecotoxicity data. At present, the lack of chronic Ni toxicity data on Arctic species constitutes the greatest hurdle toward the development of Ni quality standards in this region. Although there are some indications that polar organisms may not be more sensitive to contaminants than temperate species, biological adaptations necessary for life in polar environments may have led to differences in species sensitivities, and this must be addressed in risk assessment frameworks. Finally, Ni polar risk assessment is further complicated by climate change, which affects the Arctic at a faster rate than the rest of the world. Herein we discuss the source, fate, and toxicity of Ni in Arctic aquatic environments, and discuss how climate change effects (e.g., permafrost thawing, increased precipitation, and warming) will influence risk assessments of Ni in the Arctic.

07.08 Science and Consensus: Are They "Peas and Carrots" or "Fire and Water"?

07.08.01 Science Consensus: Pros and Cons of Conflating Science and Consensus

T. Canfield, U.S. Environmental Protection Agency / Center for Environmental Solutions and Emergency Response; W.L. Goodfellow, Exponent / Ecological and Biological Sciences; P.D. Guiney, University of Wisconsin-Madison / Molecular and Environmental Toxicology Center

Public policy is developed after consideration of multiple sources of information. When public policy is focused on the environment, science is one of those pieces that is brought into the decision process. Often the science that is included and presented is couched in a way that indicates there is a consensus among scientists agreeing with the science information that is presented and used. Through frequent use of the words science and consensus in the same discussions, it has become common to connect the two terms and present the information as "science consensus". But what does "science consensus" actually mean? Public policy decision-makers and the public come to believe that science and consensus are not only connected but often are considered the same, going together hand and glove. This is not correct as the definition of Science is "the intellectual and practical activity encompassing the systematic study of the structure and behavior of the physical and natural world through observation and experiment", while the definition of consensus is "general agreement", the judgment arrived at by most of those concerned, and/or group solidarity and sentiment and belief". But should science and consensus be used together? If so, how should they be used together? This talk will look at ways that science consensus has been used as part of public policy decision-making. We will examine the pros and cons conflating science and consensus. We will also identify areas where science and consensus may be misused when brought together. Finally, we will provide suggested guidance for how science and consensus should and shouldn't be in concert with one another.

07.08.02 SETAC Pellston Workshops - a Great Model for Reaching Consensus on a Scientific Issue

R.G. Stahl, DuPont (retired)

My first SETAC Pellston workshop was in 1993, and I've participated in several others since then. The format for a Pellston workshop is typically five days spent working in small teams to address specific questions raised on a particular scientific issue. Participants work, eat and sleep in the same location for the workshop, and often many new friendships and frameworks for future scientific collaborations are born. Yet like most consensus-building efforts, those focused on scientific issues include their share of debate, disagreements, and agreements. People will be people, whether they are scientists or not. The product of the Pellston is typically a book, but in more recent times has been a series of peer-reviewed publications in one of our SETAC journals. I've seen the Pellston workshop approach work well, and I've wondered if there are examples for successful consensus building that are not scientifically focused. In the following I will highlight the ways that Pellston workshops have proven useful for reaching consensus on scientific issues and posit on situations outside of science where consensus has been more difficult to achieve.

07.08.04 Keeping Your Partners Close and Your Critics Closer -- Tools for Integrity, Diversity, and Equity in Community Research from Full Partnerships to Contributory Projects

J.S. Kelty, Rutgers / Environmental and Occupational Health Sciences Institute

Immersion of professional researchers and community members into each other's spaces creates a more complicated and diverse mix of stakeholders and participants than research institutions with isolated decision-makers. "Community research" is a broad term used to include any research structured with responsibilities and ownership for non-professional researchers beyond a conventional study participant level. Conflicts are inevitable in these projects, especially when equitable practices diffuse decision-making power, responsibility, and accountability among supporters and critics of the research. The complexity of Integrity, Diversity, and Equity must be addressed when scientific processes push toward consensus and community change-makers interpret scientific evidence to implement actions. This presentation details the results of surveys and interviews with 100 experts in community research from around the USA addressing their challenges and best practices related to Integrity, Diversity, and Equity. Although each community is unique, some common themes were discovered across local backdrops and even across communities defined online. Perspectives of decision-makers from community, academia, government, and nonprofit organizations were incorporated. Projects spanned from full community participatory-action models (professional researchers serve as consultants to community decision-makers) to contributory models (anonymous community members provide data and resources to professional researchers). Additionally, training resources for community research teams were reviewed and curated to form a toolkit inventory. The core areas addressed in the curated trainings and tool-kits were conflict mediation, unconscious bias, shared decision making, research ethics in study design, and evolution of the community research process. Training for community-review strategies to supplement scientific peer-review will be highlighted as an example. Finally, the power of environmental community research decision-makers will be reflected in stories from the 2020 pandemic as community research teams designed to answer environmental science and justice questions pivoted into deployment of equitable pandemic solutions. Community research experts made a clear case that achieving Integrity, Diversity, and Equity improved the translational value of research, scientific quality, understanding of limitations/uncertainty, and balance of critique/consensus.

07.08.05 A Tiered Weight-Of-Evidence Risk Assessment Methodology for Informing Decisions and Drawing Conclusions About Chemical Management

C. Menzie, Exponent / Ecological & Biological Sciences; P.D. Guiney, University of Wisconsin-Madison / Molecular and Environmental Toxicology Center; S. Bellanger, P&G retired

Forrest Gump and Ginny built a life-long relationship that began with simple but important steps and grew to include more sophisticated elements over time. Viewing weight-of-evidence (WoE) through this same lens leads naturally to the idea of a tiered WoE approach. A tiered approach begins with a relationship among assessors and risk managers as they address a problem from their unique perspectives. For practical reasons, it makes sense to begin simply and to ensure there is a shared understanding. The fundamental elements of WoE are present in these initial steps. This ensures a continuum of understanding and purpose This guides the marshaling of evidence that the assessor and manager collectively understand are needed to ensure a reliable basis for decision-making. We outline a tiered WoE approach that reflects lessons learned and input from a number of workshops at SETAC meetings around the globe. Importantly, there is keen interest in reliable starting points (i.e., Tier 1) within developing countries. The approach is organized around three tiers and is designed to complement recent OECD guidance. The relationship aspects between assessors and managers are emphasized and guidance is provided on the types of information and tools that might be used at each tier. The approach also incorporates explicit feedback mechanisms to guard against unknowns. This is believed to be especially important for management of emerging chemicals. While it might be challenging to reach consensus among stakeholders around complex chemical use and management problems, the proposed tiered WoE could be the framework for achieving a shared understanding and the confidence needed to make decisions regarding acceptability, unacceptability, use restrictions, or the need for further information. WoE is more than a box of chocolates. It is the recipe for connecting science, perceptions, uncertainties, and decisions. Consensus should not be the goal of scientific inquiry but something that evolves over time despite different perspectives. It is a convergence of thought.

07.08.06 Does a "Growing Body of Evidence" Mean Consensus?

E.M. Mihaich, ER2; C. Borgert, Applied Pharmacology & Toxicology, Inc.

What constitutes a growing body of evidence that could lead to consensus? A handful of statistically significant results? A group of like-minded peers that agree with a particular interpretation of data? Reported results that have their significance overstated? In all of these cases, it isn't long before the story is told and retold and a consensus is built. But, is it meaningful, robust, reliable, and scientific? And what of replication? Replication in science is unfortunately all too rare due to limited resources and a peer review and publishing process that favors novel, flashy results over just another study showing the same thing. However, replication is crucial to the entire scientific process and to society as a whole. For consensus to be meaningful the methods used to achieve consensus must be transparent and evidence-based, the lines of evidence supporting the consensus must be reliable and replicable, and the stakeholders/scientists involved should be diverse, skeptical, and forthcoming concerning potential biases and conflicts. Case examples will be presented where "a growing body of evidence" is not as weighty as it might seem and recommendations will be offered as to how we can take better care of the scientific process as a whole.

07.08.07 Why Environmental Policy and Management Relies More on Public Consensus Than on Scientific Facts and Expertise

A. Fairbrother, Exponent / EcoSciences

Heads of government environmental agencies that manage land use, resource extraction, and chemical registration often pledge to make decisions based on the "best available science." But do they really follow through? Policy decisions are rarely based solely on rational understanding of the risks and benefits – known, unknown, and unintended – of proposed actions. This is partly due to incomplete knowledge of how environmental systems react to change, partly due to stochastic properties inherent in living systems at all levels of organization, and partly due to human-centric, value-driven environmental management goals. The biological world has no intrinsic values; it simply exists. All concepts of about what constitutes an undesirable impact and what is a desired state are derived from human experience and are influenced by cultural norms and beliefs. Policy makers inevitably must consider values outside the realm of environmental science in their decision making, such as human desires for certain lifestyle choices. Public consensus about preferred options is not always in favor of maintenance of natural ecosystems or processes. Policy debates become most contentious when scientific experts disagree on interpretation of the same set of facts and use them to support disparate positions. It is very difficult even for scientists to divorce themselves from normative biases, and frequently continue to participate in environmental policy debates not just as nonjudgmental purveyors of knowledge but also as people who purport to know the "right" answer. The scientific method consisting of controlled and repeatable experimentation was developed to help scientists reduce or resist their intrinsic biases. Yet environmental science (especially chemical risk assessment) has largely abandoned the scientific method. Rarely are studies repeated and when they are, conflicting results are ascribed to funding bias rather than to inherent variability in biological systems. Results with low statistical power are often cited as "trending towards" the desired results, and the well-known difficulty of publishing "negative" results is a strong producer of bias in the types of studies conducted. It is understandable, therefore, that public acceptance of science and expert opinion has waned, providing more space for consensus-based policy and decisions about environmental management and a reduced reliance on scientific expertise.

07.08.08 Consensorship - Has the Paradigm Been Shifted?

C. Borgert, Applied Pharmacology & Toxicology, Inc.

It has been said that science is a process wherein facts are asked to speak for themselves to enhance objectivity, and by which consensus can be achieved only as an increasingly broad and probative dataset narrows the range of interpretations consistent with the data. In this constrained vision of science, compromise and vote are anathema as competing theories vie for prominence on the merits of the evidence rather than on the agreement of affiliated practitioners. A profound and unconstrained paradigm shift may be occurring in many areas of science whereby consensus can be achieved based on normative priorities. In the unconstrained view, scientific evidence serves selectively to support the consensus and to expand the number of adherents to it. Rather than testing scientific theories by increasingly rigorous methods that winnow the range of tenable interpretations consistent with the data, this new paradigm scrutinizes those who would offer alternative interpretations of the evidence to winnow the range of tenable participants in the scientific discussion. The constrained view of science allows refinement of scientific theories according to evidence; the unconstrained view provides a basis for censoring inconvenient viewpoints irrespective of evidence, a unique development that can be labeled "consensorship." To consider whether consensorship is a valid characterization, three examples are evaluated: development, acceptance and refutation of the Linearized No-Threshold (LNT) model for ionizing radiation and chemical carcinogenesis; deployment of so-called Key Characteristics (KC) to identify endocrine disruptors and other types of toxicants; and wholesale purging of Scientific Advisory Boards based on the affiliations of the member scientists (SAB-packing). It is proposed that the LNT and KC examples share similarities consistent with a paradigm shift to consensorship, whereas SAP-packing would appear to be a political exercise rather than a scientific process, albeit one that may produce the outcome of consensorship.

07.08.09 Characterizing Diverging Perspectives of Relevance for Chemicals Policy

G. Green, J. Achar, University of British Columbia / Institute of Resources, Environment and Sustainability; G. Oberg, University of British Columbia / IRES; A. Leopold, Calidris Environment BV

Chemicals management relies heavily on science. A complicating factor is that regulatory evaluation processes commonly expect expert panels to deliver a consensus opinion. This is deeply problematic as scientific debate and dissent is central to knowledge development and there is a risk that the expectation of consensus leads to undue exclusion of potentially relevant ways of reasoning. The overarching objective of this study is to develop a method that allows identification, characterization and ultimately communication of scientific ways of reasoning that may be relevant for the evaluation of chemicals of emerging concern. We explore if a combination of bibliometrics and grounded theory can be used to identify and characterize diverging ways of reasoning in scientific documents that inform regulation of chemicals of emerging concern. Co-citation analysis was used to identify clusters in peer reviewed papers on human health effects of endocrine disrupting substances. The clusters were used as proxies for different scientific communities. Three analytical categories (tentatively called: correlation, mechanistic and categorization) were developed through an iterative and comparative content analysis of papers from the two most discrete clusters. Two distinct ways of reasoning were identified based on the way the three analytical categories are expressed. The characteristics of the two ways of reasoning will be further elaborated upon in the presentation. In a second step, the method was tested and further refined by applying it to papers on health effects of per- and polyfluoralkyl substances (PFAS). Our preliminary findings suggest that the method developed in the present study can be used to identify diverging ways of reasoning in texts informing policy about chemicals of emerging concern. The method holds the potential to support the creation of a more transparent evaluation process and reduce the risk that potentially relevant and useful scientific perspectives are being unduly excluded. The next steps will be to further validate the utility of the three analytical categories and the identified ways of reasoning by applying the method to documents produced in support of regulatory development. In addition, tool-box dialogues will be conducted with experienced expert advisors to further investigate if the identified ways of reasoning are indicative of different scientific perspectives of relevance for chemicals management.

07.09 Teaching Environmental Toxicology and Chemistry During and After COVID-19

07.09.02 Teaching Online Labs During COVID: Lessons Learned

N. Holtzman, Queens College / Biology; A. Edmond, Queens College CUNY / Biology

Queens College is a Hispanic serving institution with approximately 50% of our students receiving PELL grants. In response to the pandemic, we transitioned our introductory biology course to fully online. We remained fully online until Fall 2021. As a part of the initial transition, all the instructors were required to take a "Best practices in online teaching" course and the lab instructors worked together to create interactive, dynamic labs student can engage in, synchronously from home. Due to the financial limitations of many of our students, we use OER resources and take advantage of already available online resources such as Youtube videos and Google education suite. For our embryology lab, student are introduced to the key stages of development, define terms such as fate specification, cytoplasmic determinants and totipotency. They watch videos of embryos developing to get an overview of how each model organism develops. Then they work in groups to create a google doc or presentation of an organism. To foster inclusiveness in the lab environment, we request students have their cameras on. During the recitation, students are asked to actively engage in class discussion through directed questions. After the recitation, the lab activity is introduced and in this case. Students are placed in breakout groups to work together to complete the task. The lab instructor moves through the breakout groups to ensure students are on task and if they have any questions. The final product is a google doc or google sheet, the best of which is shared with the class by the instructor at the end of class. Each student is asked to play a specific role in the task. Efforts are made to make sure that all students are equally engaged. For the development lab, this is an exploration of different animals and why they serve as good (or not so good) model organisms. In our lab on diffusion and osmosis, we focus on the data collection and mathematical analysis of data. Preliminary analysis indicates some students flourished in this online environment and were better able to learn content since they could review the online videos and felt more comfortable asking questions. Others struggled to pay attention online and of course all learning objectives that focused on physical skills were limited. The impact of this year of online learning will only be fully assessed once students have returned and we are able to complete longevity studies on the impact of these physical skills.

07.09.03 Incorporating Experimental Design Into the Classroom: Engaging Students Outside of Research

S.A. Nutile, Penn State Behrend / Biology

Learning experimental design is an essential part of an undergraduate education within the sciences but incorporating hands-on experience for students outside of research can be difficult. Finding ways by which to incorporate experimental design within the classroom, however, can engage a diverse group of students in the process of designing and executing scientific experiments. During a 400-level Aquatic Ecology course to be taught this fall, students will be asked to design and execute an experiment related to macroinvertebrate densities on leaf packs within different aquatic habitats. Students will be presented with a variety of variables, including leaves from different species of plants and access to different water bodies, and asked to design an experiment to evaluate the effects of different aquatic habitats and leaf species on macroinvertebrate densities and decomposition rates within leaf packs. Once the experiments are complete, the students will be required to write complete scientific analysis of their results and present their experimental design and findings to the class. Results related to the perceived success of the lesson by the professor and students enrolled in the course, evaluated through pre- and post-surveys, as well as lessons learned in regard to improvements that will be made to the approach in the future will be shared.

07.09.04 Is That a Peak? Design of an Online Course-Based Research Experience that Has Students Performing a Non-Target Analysis of Dust and Consumer Product Samples

S. Kutarna, J. D'eon, University of Toronto / Chemistry

As the initial wave of the COVID-19 pandemic was spreading in the summer of 2020 and it was announced that the University of Toronto would not be opening for in-person classes in the Fall, there was an urgent need to provide students with meaningful online learning experiences. CHM 410-1410 Analytical Environmental Chemistry is a lab class that culminates in a student-directed group project where students, working in lab groups of 4-6, design and execute their own environmental analysis. While other lab activities were relatively straightforward to convert to an online format by filming demonstration videos and collecting data in advance, the group project posed the greatest challenge. This lab was our response to that challenge, as it had students performing high-level data analysis of real samples and included the risk of failure that comes with all true research experiences. This virtual lab was designed to teach students about the emerging field of non-targeted analysis by providing them with the knowledge and tools to mine existing mass spectrometry data for previously undetected compounds. The lab consisted of two sets of online tutorials designed to teach the basics of non-targeted analysis by guiding students through the detection of select organophosphate flame retardants (OPFRs) in house dust samples. Upon completion of the tutorials, groups of students were assigned a specific class of environmentally relevant compounds (e.g. per- and polyfluorinated alkyl substances, halogenated azo dyes, etc.) and each student was provided with a unique dataset from a mix of indoor house dust and consumer product samples which had not previously been analyzed for the assigned compound classes. The students in each group then worked together to synthesize their data and present a cohesive story of the chemicals detected, which they delivered in an online group presentation and individual lab reports. Student experience of the online research project was overall positive. Exit surveys conducted after submission of their final reports showed that 79% of the class felt that this lab had given them the experience of being part of a real research project. In this presentation, we will discuss the project as designed and implemented in Fall 2020, as well as our reflections on the student feedback we received and how this affected the Fall 2021 iteration of the lab, in particular how these materials were modified to incorporate in-person instruction.

07.09.05 Using Exam Reflections to Foster Higher-Level Learning in a High Enrollment Class

T. King-Heiden, University of Wisconsin, La Crosse / Biology

The shift to remote learning due to the COVID19 pandemic provided unprecedented challenges for educators. An unexpected benefit of remote learning has been the development of new tools to engage students on-line that can be implemented in our face-to-face classrooms moving forward. For me, this entailed the development of a new assessment tool that provided an avenue for students to reflect more on their learning process. I revamped an exam wrapper assignment to a more metacognitive exam reflection assignment that encouraged students to better synthesize course content and to consider the approach they took to learn. Following analysis of their multiple-choice exam, I compiled an assignment that contained several open-ended questions related to content students struggled to master along with questions asking them to reflect on their learning process. Of the students who completed the assignment after the first exam, 55% significantly improved their score on the second exam (23% improved by >10% and 7% of students improved by >20%!). All of the students who did not complete the assignment unfortunately performed worse on the second exam. Students reported that these activities helped them to take better notes in class and to better synthesize course material. While I did not note a significant change in DWF rates, I did note a shift towards higher grade distributions and overall average in the course. In the end, I was able to use multiple choice exams to identify problem areas, and the exam reflection activity to address those areas again before their final comprehensive exam. Here I will compare and contrast exam wrapper to exam reflection activities, how I implement them in my high enrollment courses, and ways I hope to promote more student interaction in the future.

07.09.06 Does Open Book Really Matter? Lessons Learned from Hyflex Teaching

A.D. Harwood, Alma College / Environmental Studies/Biology

The COVID-19 pandemic required many modifications and adaptations to our classrooms. One of the greatest challenges was how to handle cases when students had to complete assessments remotely that would normally have been monitored (e.g. quizzes and exams). One solution to avoid some cheating is to make assessments "open book/open note." In this scenario all students, regardless of if they are in the classroom have the same time and resources available to them. In order to make this effective, these assessments needed to be less reliant on memorization and more focused on application. While this strategy was completed out of necessity due to student requirements, the impact of providing students with more "cheat proof" and subsequently more applied assessments was unknown. I evaluated the impact of this type of assignments in four courses: Introduction to Environmental Studies, Global Health, Toxicology, and Environmental Health. While the courses had different proportions of "open" assessments, the results were generally the same. The grade distributions and averages were similar regardless of the changes from previous terms. Thus indicating that changing to this type of assessment did not impact overall student performance. Since these assessments can be more extensive, more applied, and more complex than those administered in a normal class period, this could be an opportunity to change how assessments are designed. Assessments in this case can be more representative of "real world" experiences and applications which could ultimately improve student learning and overall content mastery.

07.09.07 Teaching Empathy in Pharmacology: Challenges and Successes

N. Swalve, Alma College / Psychology

Addiction is a substantial public health problem in the United States and is exacerbated by things such as public health policies, environmental exposure to chemicals, and attitudes towards patients with substance use problems. One potential mechanism to decrease negative perceptions towards addiction and create policies to limit factors potentiating the vulnerability to drug abuse is through education. In spring semester of 2021, I taught a pharmacology course. The course had two primary goals: 1) increasing empathy towards patients with addiction, and 2) increasing visibility of variables that increase addiction such as environmental exposure to chemicals, genetics, and public policies. Students took a pre- and post-course survey asking about their attitudes towards public health policies related to pharmacology as well as an empathy questionnaire. Attitudes were compared after taking the course. There were changes in attitudes towards public policies after a semester of Psychopharmacology. Additionally, COVID affected the method of instruction for this course but the effects on attitudes still remained. This study suggests that education is a viable method for changing attitudes toward public policies related to pharmacology.

07.09.08 Mentoring in a Time of COVID

C. Singleman, Queens College / Center for Teaching and Learning; S. Avila, Queens College; A. Edmond, Queens College; CUNY / Biology; J. Valad, E. Fernandez, Queens College

HSI-STEM Bridges Across Eastern Queens aims to increase grades and retention in STEM for students from groups traditionally underrepresented in STEM, including Hispanic and low-income students. The project is a partnership between Queens College and its largest source of transfer students, Queensborough Community College. One main feature involves peer-based learning through a STEM peer mentoring program. Mentors attend introductory STEM classes to aid faculty during lecture or lab activities and help address student questions, as well as host office hours outside of class time. Peer mentors are trained before and during the semester in best teaching and mentoring practices. During the transition to emergency remote instruction in the spring 2020 semester, peer mentors assisted faculty by helping them use video conferencing technology, holding office hours, and meeting regularly with faculty to plan weekly online activities. During the fall 2020 and spring 2021 semesters, peer mentors continued to accommodate online instruction by assisting faculty with chats during synchronous classes and holding online office hours for students. While their tasks were accomplished differently, their prime support roles in and out of the classroom remain consistent pre- and post-COVID. Through discussions and online surveys, peer mentors report that although the transition to online learning and the continued virtual learning experience has been difficult, they have experienced positive outcomes, both personally and for their student mentees. For example, some mentors perceived that more students in their class sections joined class discussions due in part to the anonymity of participating in online courses. An increase in interactions was also clear in the number of students attending office hours. During semesters with in-person learning, the total number of students attending office hours each semester was about 52 (F19, S19, F18), During the transition period in spring 2020, 69 students attended office hours, and there was a striking increase in attendance in fall 2020 and spring 2021 with 380 and 467 students, respectively. To date, the peer mentoring program within the HSI-STEM project has shown itself to be a promising model and supportive of student learning and success. Current analysis of the data is underway to assess the broader impact of peer mentoring on student success in their introductory courses, on later courses, and on retention and graduation rates.

07.09.09 Finding the Bottleneck: How Do We Train Undergraduates in Scientific Writing?

A.M. Simpson, Penn State Erie, The Behrend College / Biology

As instructors, it is our job to teach unfamiliar concepts to students who may or may not be prepared to learn such material. Because students come from diverse educational backgrounds, it is vital to anticipate potential barriers to student understanding (i.e., “bottlenecks”). Surprisingly, students coming from distinctly different educational backgrounds may share the same bottleneck to understanding a concept, thus emphasizing the importance of diagnosing these common barriers. One ubiquitous challenge in STEM education is training students to become proficient in scientific writing. Students often struggle with the unfamiliar writing style, formatting, and organization of scientific manuscripts. Therefore, it is crucial to identify bottlenecks that are ideally shared by the entire student body. This informal study aimed to address one such bottleneck: the ability to successfully interpret and incorporate constructive feedback. A remote activity was created and assigned to first-year undergraduate students in a mixed-mode laboratory course; this activity featured a pre-written abstract that was evaluated by three reviewer archetypes (“hazy,” “rude,” and “rational”). The students were tasked with interpreting each reviewer’s comments and explaining how they would address each suggestion in the revised text. Although individual results varied within the group, the majority claimed that this activity improved their ability to incorporate peer and instructor feedback in a subsequent writing assignment. This experience suggests that this bottleneck deserves further attention from educators who teach introductory courses.

07.09.10 Impacts of COVID-19 on Graduate Students in Environmental Toxicology and Chemistry

J. Feller, Ohio State University / Department of Evolution, Ecology, and Organismal Biology; J. Damond, University of Maryland, Baltimore County / Department of Chemical, Biochemical, and Environmental Engineering; D. Green, University of Saskatchewan / Toxicology; A. Folcik, Exponent / Interdisciplinary Program in Toxicology; A. White, University of Wisconsin, Madison / Civil and Environmental Engineering

The global COVID-19 pandemic has impacted almost all facets of our lives. During the course of the pandemic, the North American Student Advisory Council (NASAC) of SETAC surveyed students within the society to see how these impacts affected their coursework, funding, research, and overall wellness. Over 170 students responded to the 27 questions in the initial survey distributed in September 2020 with a follow-up survey scheduled for June 2021. The survey was designed to give students a voice – a place where they could share their experiences, both positive and negative, and amplify their concerns and opinions to the broader scientific community. Preliminary results of the data showed strong negative trends in self-reported emotional well-being, uncertainty in funding, and concerns over support at the institutional and departmental levels. However, initial survey results also showed the strongest support coming from amongst peers and some responses highlighted the ways in which graduate students were able to adapt to the challenges and obstacles COVID-19 created. COVID-19 changed the way science is conducted at all levels and we hope that the data generated from these surveys will be useful in promoting the interests of graduate students within our organization and beyond.

07.09.11 Fantastic Failure or Surprising Success? A Discussion of Pedagogy During the COVID-19 Pandemic

A.M. Simpson, Penn State Erie, The Behrend College / Biology; A.D. Harwood, Alma College / Environmental Studies/Biology; S.A. Nutile, Penn State Behrend / Biology; C. Singleman, Queens College / Center for Teaching and Learning

The 2020-21 academic year—in the heart of the COVID-19 pandemic—tested higher education in unprecedented ways, revealing much about the functional integrity of our education system. Given that college and university administrations adopted different approaches to instruction and safety, the experiences of educators during this period were expectedly diverse. Regardless of the mode of instruction (in-person, remote, or hybrid), educators were challenged to maintain the same level of productivity and rigor in the classroom. The long-term impact of the pandemic on higher education will take time to truly understand, but that should not stop us from presently evaluating the small, yet tangible lessons that we learned this past year. Did your fool-proof, "best laid plans" go awry for unanticipated reasons? Or rather, did you find unexpected success with a hastily prepared, unfamiliar approach? Join us in a group discussion of our successes and missteps this past year!

07.10 The Little STEAM Engine that Could: How to Build, Grow, and Sustain a STEAM Outreach Program at Your Institution

07.10.01 Developing and Sustaining a Successful STEM Outreach Program

K. Witter, U.S. Environmental Protection Agency / EPA-RTP's Community Engagement and STEM Education Program; L. Bamford, Oak Ridge Associated Universities / EPA-RTP's Community Engagement and STEM Education Program

EPA's Community Engagement and STEM Education Program in Research Triangle Park (RTP), NC communicates EPA science to students, educators, and the community with the goal of increasing public understanding of how protecting the environment protects human health. Since 2003, the Program has been a model for public and private sector research organizations to leverage employee engagement and provide students, especially those from underserved communities, with meaningful STEM education and career awareness. Annually, the Program typically reaches 25,000+ people at 350+ events through the participation of 200+ employees. Outreach, both in-person and virtually, takes place at a variety of venues, including K-12 schools, universities, the community, the EPA-RTP campus, and at educator conferences and professional development workshops. Programming has historically been in central NC, with a focus on low-income schools, i.e., 50%+ free/reduced lunch, to help close the opportunity gap and build capacity for a more diverse, equitable, and inclusive workforce. This presentation will share the successful strategies and lessons learned in 18 years of developing and sustaining a STEM outreach program in a large government research organization and is applicable to both public and private sector organizations that seek to maximize employee involvement as STEM role models and advocates. We'll cover how to build relationships internally and externally, e.g., with educators, community partners, and explore how organizations can find the best fit by using employee expertise to meet STEM education needs in local schools. Characteristics of successful interactive activities that can be used to communicate organization-specific STEM messaging while meeting state science standards will be provided as well recommendations for training employees to present in classrooms, both in-person and virtually. While building a STEM outreach program is unique to each organization, we'll conclude with general principles to follow for sustaining a program, adapting to change, and sharing knowledge through educator training. These principles apply to STEM outreach programs in both the public and private sector and for organizations/programs of all sizes.

07.10.02 How Do You Pack Your STEAM Outreach Toolbox if You Have Limited Time and Budget?

W.J. Berry, U.S. Environmental Protection Agency / Atlantic Coastal Environmental Sciences Division; M. Anley-Mills, USEPA; C. Tremper, Clean Ocean Action; A. Hanson, USEPA

"You're a superhero saving our planet" – 4th grader after a hands-on STEAM activity. Come hear about practical steps you can take to make a positive and authentic contribution to Science, Technology, Engineering, Arts, and Mathematics (STEAM) education. We hope that this presentation will kick off a vibrant discussion among those individuals new to and experienced in STEAM outreach alike. If you have an interest in engaging with lifelong learners, with a little effort you can be a superhero to educators and students of all ages in your community (and further afield), too. Why is this effort important? You will improve relations with the local community, bring an awareness of your organization's scientific work, support a STEAM professionals pipeline, and bring authentic STEAM applications to life for educators and students. What's more, you will build your own skills and help advance diversity, equity, and inclusion in STEAM. But where should you start? How do you gain support at your workplace? Who can be your allies, what are some of the common challenges and how can you overcome them? What STEAM outreach resources are at your disposal? In this session we will discuss some guiding principles that have worked for us and can help you start and grow outreach efforts where you work. We will present examples of STEAM activities that we have seen work well, some of which we have developed, some we have recycled from others, and some of which we have modified from existing activities. We will cover activities that worked best in hands-on, face-to-face situations, and others that can be done virtually or via social media. If we can do this, you can do it, too.

07.10.04 Make STEAM Q: A Collaborative Project

S. Avila, Queens College; C. Singleman, Queens College / Center for Teaching and Learning; D. Wells, New York Hall of Science; M. Greco, Queens College / Art; E. Fernandez, Queens College

Make STEAM Q is a project partnering Hispanic-Serving four-year Queens College and the New York Hall of Science (NYSCI), a science museum in Queens, NY. The goal of this project is to create a community of practice featuring an interdisciplinary group of faculty who are planning and integrating "making and design thinking" activities in undergraduate courses. Drawing from complementary expertise in formal and informal learning, our team unpack their MAKER mindsets in "making STEAM" to (re)invigorate our diverse students' interest and participation in science and technology with the support from NYSCI and Queens College's Makerspace.Faculty fellows are developing courses or course modules that incorporate making and design thinking with NYSCI's Head of Maker Programming to integrate principles of informal learning based on the Design-Make-Play framework building a community of practice through best practices in andragogy, i.e. ways to teach adults, the fellows share their formal pedagogy to NYSCI to further their educational mission, and the Queens College Makerspace provides the tools and physical space for immersive learning experiences. 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. Through this collaboration, NYSCI provides a shared perspective with the Queens College mission on broadening participation of underserved students in the STEAM fields. NYSCI's programming is aimed at community engagement, providing PK-12 students and their families access to fun STEM learning experiences, while Queens College's programming aims at formal STEAM education and whose successes have been recently awarded with federally-funded education initiatives expanding undergraduate student diversity in the STEM disciplines. 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.

07.10.05 Setting the Stage for Complicated Risk Communication to an Impacted Community

S. Turnblom, Oregon Department of Environmental Quality / Western Region Environmental Cleanup; D. Farrer, T. Hudson, Oregon Health Authority; S. Turnblom, Oregon DEQ

Building trust with an environmentally impacted community is a time-consuming, long term process. This presentation will describe an interagency effort to engage a historically marginalized community affected by environmental contamination from a nearby wood treatment plant. The community has been impacted by air emissions, potential air deposition to soil in residential yards, and groundwater contamination of irrigation wells. We propose that the engagement process we describe could be successfully applied more broadly to other communities affected by complex clean-up sites or facilities where multiple agencies, programs, and environmental media are involved. Recently, a multi-agency, multi-program group engaged with interested members of the community that had been expressing their feelings of marginalization for decades. The multi-agency, multi-program group includes Oregon DEQ (cleanup, hazardous waste, water quality), Lane Regional Air Protection Agency, City of Eugene and Beyond Toxics. Since starting this community engagement group, community members have expressed feeling like they are "at the table" and not just standing by while agencies provide them with information. Factors that helped shift the community dynamic include, third party facilitation and virtual meetings that increase visibility and accessibility because no one needs to travel or find child care to attend. Community members have expressed that they feel heard. We are hopeful that this community engagement process is laying the groundwork for communities to better understanding and trust information generated by the multi-agency, multi-program group. This will be especially helpful when the time comes to communicate concepts and results that may not agree with pre-conceived beliefs held by some community members.

07.11 Utilizing Digital and Social Media for Effective Scientific Engagement in a Changing World

07.11.01 The South River Watershed- Promoting Community Engagement and Education

D. Hirschman, Hirschman Water & Environment, LLC; W.J. Reese III, AECOM / Risk Assessment; M.R. Liberati, Corteva; N. Grosso, Corteva agriscience; J. Collins, AECOM

The South River Watershed, located in Virginia (USA), encompasses approximately 230 square miles of urban, suburban, rural, and forested landscapes. Widely known for its' outdoor recreation opportunities, natural areas, diverse history of manufacturing and industry, a unique blend of cold- and warm-water fisheries, and proximity to Shenandoah National Park and the Appalachian Trail, the South River Watershed has become a destination for outdoor enthusiasts and sightseers, alike. At the center of the Watershed is the South River, where historically released mercury (Hg) from a textile manufacturing facility has accumulated on and adjacent to some bank areas, resulting in a significant source of Hg loading to biotic and abiotic media in the South River. In 2001, the South River Science Team (SRST) was created, with a composition of groups and individuals with diverse perspectives and scientific backgrounds. The SRST was intentionally established with this broad representation to achieve the ultimate goal of reducing mercury levels in fish and to promote mercury remediation strategies. As such, in an effort to reduce or eliminate Hg loading to the river, stabilization of approximately 6,000 linear feet of eroding riverbanks has been completed across several bank management areas (BMAs) with both public and private land ownership. Currently, the SRST is working with partners to develop a watershed-wide perspective to provide engaging and accessible opportunities to see the South River Watershed from a variety of perspectives, including farmers, business owners, outdoor enthusiasts, anglers, community organizations, and government agencies. As a result, the SRST's South River Current monthly newsletter and video series was developed to provide an avenue for viewers to understand basic, shared facts about the South River Watershed, including the history of Hg contamination and ongoing remediation, and to hear watershed stories in a way that inspires further interest, participation, and collaboration. This presentation will review the overall approach, considerations, and objectives of the South River Current series and will showcase, "Navigating our Watershed", the first in this series of videos.

07.11.02 Engaging Audiences with Social Media Campaigns and Open Virtual Events

C. Singleman, Queens College / Center for Teaching and Learning; E. Fernandez, Queens College

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 strengthen Queens College's identity as a minority serving institution and to help fulfill the college's motto, "We learn so that we may serve." HSI-STEM engages in standard scientific dissemination practices of presenting at conferences, writing peer-reviewed manuscripts and sharing reports with our government sponsor, but we also recognize that social media can be a powerful tool for sharing information more broadly and humanizing the scientists behind the science. Over the last year, the HSI-STEM team has worked to share the faces and stories of scientists to show the increasing diversity of scientists at CUNY. Beginning on September 15 with Hispanic Heritage Month, our social media platforms have included photos, quotations, and brief information about scientists. Many of these scientists are students, faculty, or staff at our campuses. Some are historical figures or other scientists from around the world. All of the scientists featured have been representative of the current commemorative observance. While these monthly social media campaigns have increased our social media followers each month, HSI-STEM wanted more live engagement. We held panel discussions with some of the featured members of each community and hosted seminars with invited speakers. Further, HSI-STEM collaborated with other organizations and clubs on campus to increase reach, engagement, and attendance to the events. The panels and seminars not only served to share the stories of the speakers, but also allowed for live interaction and engagement in a setting that was open to all. We saw increasing numbers of attendees with each event (all virtual this year) and each event fostered in depth and thought provoking discussions.

07.11.03 Engaging the General Public, SETAC Members and the Wider Scientific Community in Conversations About Chemicals and the European Chemical Strategy for Sustainability

M. Bloor, University of Glasgow / School of Interdisciplinary Studies; A. Leopold, Calidris Environment BV; C. Caldeira, EC JRC; J. van Dijk, Copernicus Institute of Sustainable Development, Utrecht University / Department of Environmental Sciences

Social media usage is one of the most popular online activities. In 2020, over 3.6 billion people were using social media worldwide, a number projected to increase to almost 4.41 billion in 2025. As of January 2020, the global social media usage rate stood at 49%. On average, internet users spend 144 minutes per day on social media and messaging apps, an increase of more than half an hour since 2015. Scientists are no strangers to this trend. Social media has enabled them to communicate their research quickly and efficiently throughout each corner of the world. The ability to communicate to the masses via social media is critical to the distribution of scientific information amongst professionals in the field and to the general population. However, to maximise social media engagement, the user needs to understand how the social media algorithm's function, pre-prepare curated content and establish a posting plan. In fact, even though Facebook, LinkedIn and Twitter tend to get grouped into the same general category, they are quite different. The audiences and the platforms call for slightly different styles of writing. This is one of the most significant reasons to avoid posting the same updates across the board, which can result in social media efforts falling flat. This presentation will focus on how a collaborative group of SETAC scientists embraced digital resources and social media to engage with SETAC members, the wider science community and the general public to collect preparatory information, promote and disseminate science related to the virtual 2020 #SETACSciCon Green Deal Discussion Forum, the 2021 #SETACEurope2021 Green Deal Special Session and an open access Town Hall event that followed on from it. The use of resources such as digital questionnaires, question gathering initiatives, Q codes, live polls and video makers will be discussed, but also how digital technologies and social media were used to make science accessible and start a conversation about chemicals, with a specific focus on the European Commission's Chemical Strategy for Sustainability.

07.11.04 Digital Communication After Natural Disasters - Translating the Science of Risk for a Traumatized Audience

L. Wirtis, L. Gleim, Oregon Department of Environmental Quality; M. Noble, Oregon Department of Transportation

The fires that blazed through Oregon in September 2020 were like nothing seen before in the Pacific Northwest corner of the United States – moving faster and destroying more property than any previous fire in the state. Thousands of people fled homes, many of which were severely damaged or utterly destroyed. Once the fires subsided, one fundamental question arose: when can everyone return home and begin to rebuild their lives? For the local, state and federal agencies tasked with wildfire response and recovery, answering that question required clearing several communication hurdles:Wildfire survivors had scattered across the state; some had even left Oregon to stay with friends or family. How could we communicate with people if we didn't know where they were?The fires had destroyed entire communities, rendering traditional communication tools like door hangers and postcards useless; no doors to knock on, no mailboxes to send things to.The COVID-19 pandemic added an additional wrinkle: it was still unsafe to hold any sort of in-person public meeting for communities. The fires left significant risks to human and environmental health on people's properties: asbestos, household hazardous waste like lead paint and propane tanks, and metals.The fires also left behind tens of thousands of dead or damaged "hazard trees" that could fall, without warning, on people or structures. Thousands of these trees were on private property, and posed a threat to returning homeowners. Homeowners, however, weren't ready to hear any of this; they just wanted to go home. We, as the responding state agencies, had to figure out how to translate the science, explain the risk and provide clear, accessible materials designed to keep them safe and informed. This session will summarize our approach to the first six months of communication to people affected by wildfires in the digital space: What tools worked (and which ones didn't). How to choose the right messenger.Debunking misinformation online. Communicating uncertainty. Transitioning from "response" to "recovery" in a natural disaster.How emergencies change the ways we communicate. How to build trust with people you may never meet. And we'll share our most valuable lesson learned: digital communication isn't the magic bullet that some people believe, and that even during a pandemic, finding ways to make interpersonal connections is paramount.

07.12 Policy, Management and Communication

07.12.01 Evaluating Impacts to the U.S. Department of Defense Mission From Chemical Regulation of Medium- and Long-Chain Chlorinated Paraffins

E. Williams, C. Intrator, C. Vogel, Noblis, Inc.; D. Rak, Noblis / Defense and Homeland Sec; P. Underwood, DoD

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 medium- and long-chain chlorinated paraffins (MCCPs and LCCPs) under the U.S. Toxic Substances Control Act (TSCA) and the European Union's Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation and Restriction of Hazardous Substances (RoHS) Directive. The assessment also proactively identified potential risks from increased regulation to allow sufficient time to develop and implement risk management actions. The assessment includes identifying the regulatory driver(s), gathering chemical usage data, and assessing potential impacts to the DoD. Two challenges in conducting the assessment involved (1) the complexity of categorizing chlorinated paraffins (CPs); and (2) collecting chemical and material (C/M) content information for the vast number of products and materials used by DoD and DIB. First, categorizing the CPs required tracking over 40 different Chemical Abstracts Service (CAS) numbers which are used to describe chemicals in the CP family; often these CAS numbers refer to a range of chemicals. Second, tracking C/M content required identification of data for hardware and consumable uses. To address these challenges, a literature review was leveraged to first identify an extensive list of CAS numbers and then to develop a list of secondary search terms of trade names and chemical names. These search terms were then used to query DoD data systems to locate consumable and hardware product use in weapon systems. Identified DoD uses of MCCPs and LCCPs include metalworking fluids, hydraulic fluids, sealants, adhesives, paints, and coatings. CPs often are incorporated in products for their plasticizing effect and/or to impart flame retardancy. Results presented include an assessment of potential impacts and recommendations for management actions. Relevance of this assessment extends beyond CPs 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.

07.12.02 Fluidity of Science: A Misunderstanding that Is an Obstacle in Communications

L. Kapustka, LK Consultancy

Communicating science about issues of societal relevance, especially if there are policy implications is challenging. Tropes such as "settled science" create expectations of un-fallibility. Yet, if we adhere to the clear tests of science that were provided by Judge William Overton in his 1982 ruling in McLean v. Arkansas Board of Education, we must acknowledge that to qualify as science, a conclusion must be falsifiable. As social-ecological system dynamics come into play in policy debates, it is desirable to achieve consensus among the affected stakeholders. The cautionary element that members of the scientific community can contribute is to be humble about what we know and what we don't know. What we know, in some situations could be refuted as new information emerges. Communicating the fluidity of science with an apparent innate tendency to hold onto old positions confound efforts to reach consensus. The economist John Kenneth Galbraith opined, "Faced with the choice between changing one's mind and proving that there is no need to do so, almost everyone gets busy on the proof." Examples of science fluidity will be offered to advance this case.

07.12.03 Nanomaterials in the European Chemicals Legislation - Methodological Challenges for Registration and Environmental Safety Assessment

M. Nielsen, A. Baun, A. Mackevica, Technical University of Denmark / Environmental Engineering; A. Thit Jensen, Roskilde University / Science and Environment; I. Odnevall Wallinder, KTH Royal Institute of Technology; J. Gallego, University of Gothenburg / Department of Marine Sciences; L.P. Clausen, Technical University of Denmark / Department of Evironmental Engineering; J. Rissler, RISE Research Institute of Sweden / Department of Material and Surface Design; L. Skjolding, DTU / DTU Environment; A.C. Nilsson, SOLVE Research and Consultancy AB; T. Cedervall, Lund University / Chemistry; S.F. Hansen, Technical University of Denmark / DTU Environment

In the European Union the Annexes of its chemical legislation (REACH) were revised and now clarify the technical data requirements for nanomaterials (NMs). These new provisions, effective from January 1, 2020, introduce requirements for manufacturers, importers and downstream users regarding registration and safety assessment of NMs. This study aims to assess the availability and suitability of methods needed to comply with the new regulatory provisions on NMs for physico-chemical characterisation and environmental fate and effects. The scientific literature and relevant test guideline frameworks were reviewed to identify applicable methods. These were subsequently evaluated and categorised as either: 'internationally accepted test guideline or standard (TGS)', 'internationally accepted test guideline or standard under development (TGSUD)', 'established as standard methods in scientific literature (SCI)', 'other methods and/or more research needed (O)' or 'no method (N)'. We find that 80% of the information requirements and a bit more than 40% of the waiving criteria in the new REACH Annexes are supported by methods that are available as TGS, TGSUD or SCI. Most of the relevant methods in the scientific literature are included in recent OECD guidance documents or ECHA guidance. We recommend that a targeted effort is made to develop protocols and guidelines for methods to determine NM adsorption/desorption, degradation, exposure scenarios and ability to cross biological membranes. Here methods to fulfil the information requirements and waiving criteria are currently lacking. Furthermore, we recommend that increasing attention is directed towards regulatory reliability and relevance of the information that is submitted by the registrants.

07.12.04 Next Generation of European Nanomaterial Regulation - the Way Forward

M. Nielsen, L.P. Clausen, Technical University of Denmark / Department of Evironmental Engineering; S.F. Hansen, Technical University of Denmark / DTU Environment

The unique properties and multiple application possibilities of nanomaterials has led to a rapid development in the field of nanotechnology since the 2000s. Concerns have been raised about the potential risks that nanomaterials might pose to human health and the environment, due to the inevitable release of nanomaterials from the increasing production and societal use. In Europe, numerous health and environmental regulations exist to secure a safe production and use of chemicals and consumer products. There has been a substantial increase in the adoption of nano-specific provision in these regulations in recent years. However, there is a need to assess the sufficiency and suitability of the regulations to incorporate nanomaterials. The purpose of this study is therefore to analyze and compare key features of European regulations relevant to nanomaterials, identify strengths and weaknesses, and provide recommendations for future efforts to overcome identified regulatory challenges related to nanomaterials. We used Eurlex to identify relevant legislation and for each Regulation, Directive etc. identified as relevant, an in-depth analysis was conducted with regard to key definitions, information requirements, risk management procedures, monitoring requirements and strengths and limitations. The analysis showed that many regulations have been revised to explicitly cover nanomaterials. Limitations evidently still exist, despite of these efforts. Specific challenges exist for the individual regulations and include among others challenges in regard to grouping of nanoforms in the European Chemical legislation, identification of treated articles in the Biocidal Product Regulation, challenges for pre-market safety assessment in the Regulation on Cosmetic Products, inadequate hazard categorization of nanomaterials in the EU Ecolabel Regulation and uncertainties with establishing environmental quality standards for nanomaterials in the EU Water Framework Directive. Cross-cutting limitations include the lack of a common nanomaterial definition among the regulations, unclear terminology used in nanomaterial definitions and lack of certain nano-specific test methods. Based on our findings, we recommend to develop suitable test methods where these lack, unify the nanomaterial definition, allocate more resources to enforcement and validate if the principles for deriving Environmental Quality Standards for chemicals can be transferred directly to nanoparticles.

07.12.06 Sewage Surveillance Modelling and Communication of SARS-CoV-2 Risk in Michigan

C. Laba, J. Hart, Oakland University / Chemistry; D. Szlag, Lake Superior State University / Chemistry

We describe a comprehensive Sewage Surveillance (SS) program in Michigan that provides a complimentary strategy to clinical testing for assessing and communicating the prevalence of COVID-19 infections in congregate facilities such as senior living and nursing homes as well as communities via analysis at wastewater treatment plants. The cornerstone of this program is a cost-effective robust workflow based on a network of autosamplers, PEG precipitation, and molecular quantification of SARS-CoV-2 viral targets using a CDC qPCR detection method adapted to a droplet digital PCR (ddPCR) analysis platform. The SARS CoV-2 targets are amplified using one-step reverse transcriptase and polymerase and CDC recommended primer and probe sets for N1 and N2 as well as the E targets. A persistent question in SS is which targets to use and how many targets are needed to identify the presence SARS-CoV-2. That question is a complicated by many factors, but analysis of over 2000 data points showed that the N1 and N2 targets were equally likely (~85-90%) to detect low concentrations of viral marker concentration as compared to the E target (~75%), and when combined they could account for over 97% of the detections. For a SS program to be actionable and successful, it is paramount that sewage surveillance data be reported rapidly and communicated a form, useful to decision makers. Raw SARS-CoV-2 PCR data must be normalized to be actionable. In our Michigan study, we built a model and dashboard that brought together information on population, at-risk population, clinical cases, and sewage velocity and temperature. Surprisingly, kinetic studies showed that degradation of the N1, N2, and E targets was quite slow relative to travel times in our systems and that the target sequences were quite stable even at elevated temperatures. Most importantly we show that with normalization and flow modeling, the SS targets lead the clinical case data by 3-7 days and can be used to optomize clinical testing and other public health measures. We also briefly describe our observations with SS variant testing and our experiences with several different variant testing workflows.

07.13 A Shared Sense of Place: Indigenous Perspectives on Motivating Communities to Respect the Environment

07.13.02 Developing, Collating and Sharing Environmental Research Protocols for Tribal Nations

L. Bruce, Gila River Indian Community; S. Gaughen, Pala Band of Mission Indians; N. Patterson, SUNY College of Environmental Science and Forestry; B. Rashleigh, U.S. Environmental Protection Agency; J. Zambrana, U.S. Environmental Protection Agency / Office of Research and Development

The U.S. Environmental Protection Agency (EPA) works directly with Tribal Governments in protecting human health and the environment in Indian Country. EPA also works with Tribal Partnership Groups such as the EPA National Tribal Science Council (TSC) and the EPA Region 9 (Southwest) Regional Tribal Operations Committee (RTOC) to assist EPA in meeting its trust responsibility to the Tribes, enhance EPA's responsiveness to Tribal needs, and provide a forum for interaction and communication between Tribal and Agency representatives of mutual benefit and responsibility to work collaboratively on environmental scientific issues. One activity started by the TSC and then taken up by the R9 RTOC is collecting examples of indigenous research frameworks, protocols and methodologies. Past infringements of Tribal sovereignty have made protocols for research on Tribal lands critical. Tribal research frameworks and policies for engagement with non-tribal scientists are expressed in many ways, including: Tribal Resolutions, Research Agreements, Data Management Plans, Quality Assurance Project Plans, Cultural Committee Review Processes, Institutional Review Board (IRB) Agreements, Tribal College and University IRBs, and Non-Disclosure Agreements. The purpose of this collection effort has been multifold: to better understand data and research needs from a Tribal community perspective; to identify examples of data partnerships between research institutions and Tribes; to better ensure that researchers center Indigenous knowledge in addition to decentering Western colonial frameworks; and to collate information to serve as a repository. This presentation will highlight the work of the TSC and R9 RTOC is doing to collect and share examples of Tribal protocols for researchers, and to develop a template that Tribal Nations can adapt to develop their own protocols. Information to be collected and shared more broadly includes history or information on research with Indigenous communities, legal cases or briefs related to research process or outcomes, and specific Tribal examples of research frameworks or resources. Key items the template will highlight include asserting Tribal sovereignty, protecting Tribal knowledge, centering Tribal research priorities, controlling access to Tribal data, benefiting Tribal communities, and supporting ethical research in Tribal communities.

08.01 Advancements in Life Cycle Assessment

08.01.01 Net Zero in 2050: Steering Greenhouse Gas Reductions in the Chemical Industry by Implementation of a Comprehensive and Scalable Digital Tool

P. Saling, V. Silva, A. Pistillo, J. Schoeneboom, BASF SE / Corporate Strategy and Sustainability

Chemistry is an enabler of many solutions to provide more sustainability for societies and to support them to achieve the goals for climate change being net zero in 2050. To make the contributions of chemicals along value chains transparent and visible it is needed to generate meaningful data supporting decision-making processes. In this context, the Chemical Industry has a huge challenge: to become climate-neutral by 2050 as well. To achieve the net-zero greenhouse gas emissions is at the heart of the European Green Deal and in line with the EU's commitment to global climate action under the Paris Agreement. This ambitious target creates several opportunities for the chemical industry to contribute for a better future. Steering decarbonization requires full transparency of the carbon footprints along the value chains to identify the opportunities and for that a standardized methodology for Product Carbon Footprint (PCF) calculation at scale must be established. BASF builds on 25 years of experience in methodologies to quantify sustainability performance and this paper aims to define the methodological principles and product specific guidance for the calculation of a cradle-to-gate PCF following ISO standard 14067:2018 for carbon footprint of products, which is based on ISO14040:2006 and 14044:2006 for life cycle assessment and, additionally, aligned with the GHG Protocol Product Standard (WRI & WBCSD, 2011). Practical examples about the bottom-up ("LCA approach") calculation, data consolidated along the specific cradle-to-gate process-network of each product, including the decision tree logic for multi-output processes where allocation is needed, and data automation approaches will be presented. It will be shown a new approach that enables industry to achieve the automated annual calculation approach of cradle-to-gate Product Carbon Footprints. BASF has established a system for more than 45,000 sales products, produced at 700 plants, through about 450,000 processes, using about 20,000 raw materials. It will be discussed with the audience how that approach can be implemented by other companies to generate a powerful data exchange format. The development of the digital application revealed that there is a need for high-quality input data as well as for a standardization of the calculation method within and beyond the Chemical Industry.

08.01.02 Exploring Human Behavior in Life Cycle Assessment - Agent Based Models as One Option

A. Hicks, University of Wisconsin, Madison / Civil and Environmental Engineering

Humans interact with the products they use, which in turn influence the life cycle environmental impacts of those products. Life cycle assessment (LCA) is a critical tool in assessing the environmental impacts of products and processes. At the same time, it is not well suited on its own to exploring the shifts in the anticipated environmental impacts of a product or process due to human behavior. Agent based modeling (ABM), a bottom up modeling framework, is well suited to explore human behavior. Through coupling LCA and ABM there is the opportunity to explore the influence of human behavior, and see the people, in these studies. The potential influence of human behavior at the different product life cycle stages is presented utilizing grocery shopping bags as an example of the potential for humans to shift from the idealized systems we often utilize in LCA. Beyond that, the coupling of these two tools has the potential to lead to more accurate forecasts of the environmental impact of products and processes where human behavior has a significant impact, such as during the adoption or use phases.

08.02 Alternatives Assessment and Informed Substitution: The Core of a Safer Chemical Economy

08.02.01 Progress in Alternative Assessment Frameworks: The Guidance on Key Considerations for the Identification and Selection of Safer Chemical Alternatives

L. Duffy, U.S. Environmental Protection Agency / Region 1; E. Connor, Abt Associates / Environment Resources Division; E. Leinala, OECD; C. Davies, U.S. Environmental Protection Agency; M. Jacobs, University of Massachusetts, Lowell / Lowell Center for Sustainable Production; J. Tickner, University of Massachusetts, Lowell / Community Health and Sustainability; D. Mottet, ECHA / Risk Management Implementation Unit

The alternatives assessment field is becoming increasingly important in guiding the transition towards safer alternatives. The use and practice of alternatives assessments have advanced considerably in the last decade; however, an important limitation that can hinder efforts to identify alternatives for priority chemicals is the lack of consistent criteria for defining "safer." Safer, in this context, refers to a chemical, product, or technology that is preferable, in terms of both hazard and potential for exposure to humans and the environment, than the existing option. The Organisation for Economic Co-operation and Development (OECD) published a guidance in 2021 to advance a consistent understanding of the minimum requirements needed to determine whether a chemical alternative is safer than the priority chemical, product or technology for substitution. The guidance recognized these minimum requirements are the baseline level in a spectrum of an increasingly comprehensive set of criteria and assessment practices that may be needed to provide confidence that a given alternative is safer. To identify safer alternatives, the guidance recommended excluding alternatives that are classified as "high" concerns based on Globally Harmonized System (GHS) criteria for carcinogenicity, mutagenicity, reproductive and developmental toxicity, persistent bioaccumulative and toxic (PBT) chemicals, and very persistent and very bioaccumulative (vPvB) chemicals for the comparative hazard assessment step. The guidance then recommended performing a qualitative exposure assessment comparing the exposure potential of alternatives relative to the chemistry being replaced. This could be accomplished by using available data or physical-chemical properties. A safer alternative determination requires integrating the results of the hazard and qualitative exposure assessments to evaluate trade-offs and consider any lingering uncertainties. A broadly agreed upon approach for this integration step does not exist so the guidance focused on the need to be transparent throughout the assessment process and to engage stakeholders. This guidance advances the field of alternatives assessment by formally documenting broadly agreed upon minimum criteria and practices, while not precluding assessors from including more comprehensive approaches in their alternatives assessments. *The views expressed in this abstract are solely those of the authors and do not represent the policies of EPA.*

08.02.02 Building Capacity for Robust Pesticide Regulation Abstract

T. Malloy, University of California, Los Angeles / Law; P. Allard, University of California, Los Angeles / Institute of Society and Genetics; M. Isied, University of California, Los Angeles / IoES

California state law establishes elaborate state and county programs intended to minimize the human health and environmental impacts of pesticide use. Despite the many strengths of these programs, recent scientific and legal evaluations of them called for improvements in the availability of potentially safer alternatives to hazardous pesticides. This research aims to facilitate development of alternatives assessment (AA) methods fit for use in the context of California's state and county regulatory programs. We use a mixed method approach, including (1) a review of academic literature and grey literature regarding existing and emerging methods and tools, (2) interviews of experts in methods and representative stakeholders regarding potential methodological approaches in the pesticide regulatory context, and (3) an action-oriented workshop convening scientists, policy experts and stakeholders to critically evaluate potential AA frameworks. The presentation will explore a set of methodological frameworks and tools that could be used for AA in state and local regulatory programs, highlighting benefits, limitations, and resource needs.

08.02.03 West Coast States Taking Different Approaches to Alternatives Assessment

T. Lewandowski, J. Zhang, Gradient; A. Yeh, University of Washington; J.M. Cohen, Gradient

While various jurisdictions around the world are mandating and conducting alternatives assessment (AA), activity among states on the US West Coast has been particularly notable, both in terms of the level of activity and the differences in approaches being implemented. California's Safer Consumer Products (SCP) program is both the earliest and most comprehensive, but the highly detailed nature of the State's AA requirements and the need to designate individual products through formal rulemaking has limited the number of products that have actually moved through the process. In contrast, AA under Oregon's Toxic Free Kids program, coming into effect in January 2022, is much more narrowly tailored to certain children's products and provides a number of "off ramps" for products prior to the AA requirement. Unlike California, any product falling into certain designated product classes is automatically subject to the regulation, so a large number of products could theoretically move through the process at the same time. Like California, manufacturers will conduct the AA, but reviews will be conducted by third-party assessors under the State's direction. Unlike California, Oregon's program relies on pre-existing AA and hazard assessment frameworks. Washington state's AA program has features in common with each of the others; for example, it has a more limited set of AA requirements like Oregon (both using the IC2 AA framework) but can address products used by any population of concern, as is the case in California. Unlike the other two programs, Washington's requires the State to conduct the AA and regulated parties are generally limited to a commenting role. The talk will compare and contrast the three programs, commenting on the advantages and disadvantages of each and the challenges each program may pose for the regulated community. Comparisons to other regulatory jurisdictions (*e.g.*, Europe under REACH, other US states) will also be offered where relevant. This talk will review the key features of these three programs to understand their similarities and differences.

08.02.04 Safer Products for Washington: Identifying Safer, Feasible, and Available Alternatives to Priority Chemicals in Consumer Products

M. Smith, C. Manahan, S. VanBergen, S. Stump, R. Eaton, L. Tamboer, Washington State Department of Ecology; H. Davies, E. Fanning, Washington State Department of Health; C. Niemi, Washington State Department of Ecology

Many consumer products we use in our daily lives contain toxic chemicals. Once hazardous chemicals are in consumer products, reducing exposure is challenging. It's hard to predict how people will use consumer products and what they'll do with them when they're done. Contamination from hazardous chemicals in consumer products can affect consumers, communities, wildlife, and environmental resources. In fact, consumer products are one of the largest sources of toxic chemicals in our environment. 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. Instead of conducting single chemical and product risk assessments, Safer Products for Washington focuses on reducing the use of classes of hazardous chemicals by moving to safer alternatives. This approach gives us the opportunity to reduce the impacts of hazardous chemicals across the product lifecycle—from manufacturing to disposal or reuse. It means we focus on pollution prevention instead of costly clean-ups. The law requires us to identify priority chemicals and products and then make regulatory determinations based on whether safer alternatives are feasible and available, among other criteria. The first set of eleven priority product-chemical combinations are: · Per- and polyfluoroalkyl substances in carpets and rugs, aftermarket stain treatments, and furnishings. · Polychlorinated biphenyls in paints and printing inks. · Phenolic compounds in laundry detergents, can linings, and thermal paper. · Flame retardants in recreational foam products and electric and electronic enclosures. · Phthalates in vinyl flooring products and personal care and beauty products. This presentation will describe our criteria for identifying safer, feasible, and available alternatives, as well as preliminary findings for our first set of priority product-chemical

08.02.05 Themes From Evaluating Regulatory AAs From California's Safer Consumer Products Program

K. Grant, Safer Products and Workplaces Program / Safer Consumer Products; T. Buck, Northeast Waste Management Officials Association (NEWMOA) / Interstate Chemicals Clearinghouse (IC2); L. Wong, M. Romero-Fishback, Q. Meng, J. Baker, H. Lee, Safer Products and Workplaces Program / Safer Consumer Products; X. Zhou, California Environmental Protection Agency / Department of Toxic Substances Control

California's Safer Consumer Products Program (SCP) mandates that manufacturers of certain problematic product-chemical combinations, termed Priority Products, conduct Alternatives Analyses (AAs) to identify safer chemical ingredients. While the program has received AAs on two Priority Products, some potential trends have surfaced. Since these are among the first regulatory AAs in the U.S., it is important to share some of the themes and challenges from SCP's evaluation of the AAs. One theme is that manufacturers focused on a limited scope of alternatives. Additionally, the manufacturers sought a single replacement for several different functional use situations, rather than considering a safer product that could accomplish a particular task. For example, methylene chloride paint stripper manufacturers preferred alternatives that could achieve the highest performance standards, such as stripping automotive paint, while safer alternatives are already on the market that easily strip latex paint off wood. The economics section of the SCP AA poses another challenge. This section is intended to be broader than just examining the cost to the manufacturer. Instead, it is supposed to account for the externalities associated with the continued use of the chemical of concern. This information proved difficult to find. The first AAs were submitted by consortia; we will discuss some of the benefits and challenges that presented. We will provide an overview of these themes and preview challenges and opportunities with upcoming AAs that may be applicable to the entire AA community.

08.02.06 Lessons Learned From Conducting Alternatives Analyses Under California's Safer Consumer Products Program

T. Lewandowski, J.M. Cohen, J. Zhang, Gradient

California's Safer Consumer Products (SCP) program mandates alternatives assessments (called in this case alternatives analyses) for products the State has designated as priority products (*i.e.*, certain products containing chemicals of concern with potential for widespread exposure). To date, alternatives analyses (AAs) for two priority products have been completed: paint removers containing methylene chloride and two-part spray foam insulation systems containing unreacted methylene diphenyl diisocyanate. This presentation will offer key lessons learned in conducting these first AAs under the SCP program. We will discuss challenges we encountered in evaluating dozens of products and possible alternatives, many of which have lacked data on performance, hazard, or cost. There have also been challenges in defining an alternative (*i.e.*, what types of technologies to consider, uncertainties regarding the degree of exposure reduction that would be meaningful). A related issue is the requirement to consider product patent applications where the viability and specifics of the possible alternative may be hard to gauge, examples of which will be provided. One unique aspect of the SCP program is the large number of relevant factors (over 100) that must be addressed in conducting the AA, ranging from human health toxicity to surface water quality impacts, from raw materials depletion to third party health care costs. We will discuss strategies for keeping the level of effort in evaluating these manageable. We will describe how a quantitative hazard ranking scheme was used to numerically compare products and alternatives, including methods to account for data gaps. Finally, we will discuss the decision-making process and how different approaches (from the purely qualitative to purely quantitative) can be used in making decisions about a product. We will conclude with several key recommendations for carrying out AAs under the SCP program and suggestions on how the program could be improved to be more productive.

08.02.07 Helping Massachusetts Companies Replace Halogenated Solvents with Safer Options

P.A. Eliason, H. Hudson, Toxics Use Reduction Institute

The Toxics Use Reduction Institute has been working with Massachusetts companies for over 30 years to help them reduce their use of toxic chemicals. Solvents comprise the highest volume of toxic chemicals used by Massachusetts manufacturers. The majority of these uses are for cleaning industrial parts within or at the end of the manufacturing process. Halogenated solvents have historically been the most common type of solvent as they perform well with a variety of contaminants on a wide range of substrates. The TURI Lab has extensive experience working with companies to identify safer solvents that perform well for their specific applications. To aid in the identification of these solvents, the Lab created a database (www.cleanersolutions.org) of the performance of safer alternatives, which incorporates relative hazard information derived from the Pollution Prevention Options Analysis System (P2OASys). TURI created a guide to help companies identify safer and effective alternatives to their unique use of halogenated solvents on their own. The guide provides tips for considering relevant trade-offs when assessing alternatives. We will describe the guide and the tools used. This will be done in the context of several case examples of companies that have changed their processes to eliminate the use of halogenated solvents. The drivers for change differ for each example, but the need to deliver sufficient performance is the key criterion. These case examples will include a manufacturer of hot runner systems for the plastics injection molding industry that replaced a 1-bromopropane vapor degreaser with an alkaline aqueous cleaner, a manufacturer of ceramic feed throughs for medical and aerospace applications that replaced TCE with a heated aqueous system that contains 1% borax and baking soda, a technical high school that replaced its automotive parts washer and aerosols with a biobased system, and a manufacturer of micro-drilled parts for precision glass jewels that replaced TCE with an ester-based solvent alternative. Unexpected benefits of replacing halogenated solvents will also be discussed.

08.02.08 Informed Substitution of Pesticides: A Two-Tier In Silico Approach for Design of Photodegradable Pesticides with Minimal Ecotoxicity

J. Lewer, J. Huang, J. Peloquin, Z. Stickelman, J. Kostal, The George Washington University / Chemistry

Elucidating factors that contribute to ecotoxicity and persistence of agrochemicals in the environment is necessary in developing safer alternatives for tomorrow's pesticides. Chromophoric Dissolved Organic Matter in the excited triple state (^{3}CDOM*) is known to play a key role in the removal of pesticides via indirect photodegradation. Here, we report a two-tier computational framework developed to probe and predict both kinetics and thermodynamics of ^{3}CDOM*-pesticide photodegradation. In the first tier, robust *in silico* models were constructed by fitting free energies obtained from density functional theory (DFT) calculations to cell potentials and second-order rate constants for the ^{3}CDOM*-pesticide electron transfer. In the second tier, calculations based in Frontier Molecular Orbital (FMO) theory were fit to electron-transfer energetics and applied across the entire USEPA's pesticide registry; effectively leveraging computational methods to circumvent experimental data gaps. In conjunction with previously developed FMO metrics for ecotoxicity, we present a novel tool which integrates key criteria for environmental safety into structure-based design. Being highly mechanistic and based on ca. 1,500 unique ^{3}CDOM*-pesticide interactions, this method represents a robust approach for substituting hazardous pesticides with safer alternatives. To that end, we outline the development and performance of our model as well as its incorporation into an open-source Pesticide Indirect Photodegradation (PIP) platform. Housing all computed and experimental data, PIP can be used to assess alternative pesticides for environmental safety, as well as guide the design of safer chemicals by integrating SMARTS-based substructure-matching and Tanimoto-coefficient similarity scoring.

08.02.09 Quantitative Hazard Assessment and Product Scoring Methods as a Scalable Approach to Identification and Selection of Safer Chemical Alternatives

C.E. McLoughlin, SciVera LLC / Toxicology; P. Beattie, L. Bestervelt, J. Rinkevich, SciVera LLC

In March 2021, OECD released a report entitled "Guidance on Key Considerations for the Identification and Selection of Safer Chemical Alternatives" with the goal to "advance broader agreement on a general approach and criteria for the selection of safer alternatives, with a focus on chemical substitution." The authors will present recent work on tools in use throughout chemical supply chains by chemical suppliers, product formulators, textile mills, brands, and retailers transforming the way chemical selection is occuring for consumer products. Chemical hazard assessments (CHAs) are conducted using the GHS+ methodology which is based on 23 human and environmental health endpoints and physical characteristics. GHS+ CHAs provide an innovative combination of Qualitative and Quantitative Comprehensive Hazard Assessment (QQCHA) methods including the Scivera Index. Safer alternative selection is aided by functional use groups (FUGs), chemical and formulation scoring, and integrative methods for downstream knowledge, all of which are made possible by computer software. The textile industry is at the forefront of the movement towards safer chemical identification and selection (including upstream safer chemical screening, assessment and scoring). One highly successful program for chemical assessment and product scoring is Screened / Scored Chemistry (SC). SC requires Confidential Full Formulation Disclosure (CFFD) in textile formulations. The authors have screened over 800 formulations with 100s of chemicals under SC and results show progress throughout the supply chain in achieving selecting safer chemistries. Instant availability of QQCHA results for over 4,000 commonly-used chemistries, all reviewed by board-certified toxicologists, dramatically reduces the cost of safer chemistry exploration and selection with thousands of additional instant results for unacceptable or potentially safer alternatives available for formulators to explore. "The guidance focuses on minimum assessment criteria and recommended assessment practices for four core areas of alternatives assessments: (1) determining assessment scope, (2) comparative hazard assessment, (3) comparative exposure assessment, and (4) the integration of hazard and exposure results to identify safer alternatives." The authors will discuss how GHS+, Screened Chemistry, and software support the OECD guidance including "moving beyond the minimum''.

08.02.10 High Throughput Ingredient Screening to Improve the Sustainability Profile of a Portfolio of Consumer Products

A.J. Conway, Integral Consulting, Inc.; P.C. DeLeo, Solleone Consulting, Inc.; S. Wang, C. Ross, Integral Consulting, Inc.; A. Carrao, Kao USA / R&D; A. Dysert, Kao USA

Consumer product manufacturers are keenly aware of their customers' desires for products that perform well but also have minimal impacts to the environment. Increasingly, product manufacturers are incorporating quantifiable metrics to improve the safety profile of their ingredients into their environmental, social and governance (ESG) goals. One way to minimize the environmental impacts of a formulated consumer product following end-of-use disposal, is to use ingredients that have both low ecotoxicity and are rapidly biodegradable. The ingredient portfolio for a major global manufacturer of formulated consumer products was analyzed to identify priorities for substitution. In order to carry out this assessment, ecotoxicity and biodegradation data were compiled using published data retrieved from the European Chemicals Agency dossiers, various U.S. Environmental Protection Agency databases, and other reputable sources for over 200 ingredients. When experimental data were not available, in silico techniques were utilized to identify similar compounds (analogues) whose experimental data could be applied, or quantitative structure-activity/property relationship models (QSAR/QSPR) were applied. We have outlined the results of this data compilation and presented groups of compounds with higher toxicity (LC50 or EC50 < 1 mg/L), lower biodegradation (not like to be biodegradable), or a combination of the two to identify areas where improvements may be made in the environmental safety of formulations.

08.02.11 Looking Beyond the CAS Number: Considering Form Specificity in Hazard Assessment of Metals

C. Marsh, K. Reynolds Reid, Gradient; C.A. Claytor, Copper Development Association, Inc.

Hazard evaluation frameworks and alternatives assessment (AA) tools are deeply rooted in the globally harmonized system for classification and labelling of chemicals (GHS). Often, existing GHS classifications and other hazard-based reference lists developed by regulatory and advisory agencies are also factored into these assessment schemes. Metal substances, such as copper, are often produced and used in distinct physical forms including powders and massive or "bulk" forms. Although these forms share the same Chemical Abstracts Service (CAS) number, the physical properties of these distinct forms often differ widely and drive the physical, human health, and environmental hazards, such that a GHS evaluation of copper powder and copper massive results in a distinct set of hazards for each form. While the GHS guidance addresses form specificity of metals as it relates to aquatic toxicity, guidance for other toxicity endpoints is largely left to "expert judgement". Moreover, many existing hazard assessment frameworks, databases, and tools such as Pharos, GreenScreen®, Toxnot, and Health Product Declarations (HPDs) are largely organized by CAS number, which can present potential challenges for data organization and hazard communication. For example, existing hazard lists for a single CAS number may only apply to a particular form, but this information may not be readily apparent to an assessor or data user. To demonstrate the associated challenges, we are presenting a case study in which we evaluated copper powder (< 1 mm) and copper massive (≥1 mm) using both GHS and the GreenScreen® method. Technical issues related to assigning form-specific hazards and opportunities to address challenges within current assessment tools are described.

08.02.12 Advancing High-Throughput Environmental Risk Assessment of Fragrance Materials to Improve Transparency and Enhance Safe Use

P.C. DeLeo, Solleone Consulting, Inc.; C. Stevens, Integral Consulting, Inc.; A. Lapczynski, RIFM / Environmental Science

For more than 50 years, the Research Institute for Fragrance Materials (RIFM) has led programs to demonstrate the human and environmental safety of more than 3,000 fragrance materials manufactured and used by their members. RIFM sponsors testing and academic research on the fate and effects of fragrance materials in aquatic, soil and sediment compartments. In addition, RIFM conducts environmental risk assessments and screens fragrance materials using the RIFM Environmental Framework published almost 20 years ago (Salvito et al. 2002. *Environ. Toxicol. Chem.*, 21: 1301-1308). While the Framework has proved to be an effective tool, its scope was limited to the United States and Europe. RIFM recognized the need to expand the geographic scope of those assessments to include regions with rapidly expanding usage of fragrance materials. Also, advances in environmental exposure science and ecological hazard characterization over the past two decades offer an opportunity for the Framework to showcase cutting edge science and data analytics. This presentation will highlight recent advances of the Framework that feature expanded geographies, real-time access to the most current population and hydrological data, mechanism of action material categorization and the ability to reassess the entire inventory of fragrance materials nearly instantaneously at the push of a button. The updated Framework represents an enhanced risk assessment tool for RIFM as well as a tool for fragrance material suppliers and users to make better formulation decisions.

08.02.13 Assessing Alternatives in View of Uncertainty in Toxicological Data: New Approach Methods (NAMs)

H. Plugge, Safer Chemical Analytics, LLC

Greener chemical alternatives most likely will not have classical toxicology data but NAMs data instead. Comparing health effects between a compound of concern and an alternative thus involves very different methodologies and inherent uncertainties. Hazard assessment as part of an Alternatives Assessment (AA) is based directly on all health (and ecological) effects used in a screening effort. NAMs offer a relatively inexpensive, quick, and ethical opportunity to screen hazards. Positive results/flags would result in elimination of alternatives. Negative results move an alternative forward from an AA to eventually a regulatory submission. Although existing conventional toxicology data are thought of as "gold" standards, they are much less certain than previously assumed. Recent studies have found that inherent data uncertainties are in the ± half order of magnitude range at best and can easily range two orders of magnitude for e.g. fish. These uncertainties already imply that categorization/classifications can span two or more categories. In addition, NAMs can have better concordance and repeatability as compared to animal data. NAMs thus only have to be as good as traditional data, better would be a bonus. Using an actual biocide evaluation for isothiazolinones, the uncertainty in hazard assessment using NAMs will be described. These chemicals are meant as less toxic alternatives to existing biocides. The hazard assessment of the skin sensitization potential uses a NAM composed of three separate tests in a Defined Approach. Hazard identification did not result in a "flag". Risk assessment of this group of chemicals (isothiazolinones)' skin sensitization potential via a NAM included an uncertainty factor of 10,000 despite the NAM's concordance with human data (much higher than animal models). Based on these hazard assessment data (leaving aside the exposure considerations), the alternatives would incur at least a 100 fold "penalty" as compared to the compounds of concern, even though they passed the hazard assessment phase. From a regulatory point this has the potential for great inequities – an alternative that has been assessed via NAMs gets hit with an extra (>= 100) fold assessment factor: indicating the regulator/assessor is not comfortable with the NAM methodology. The likelihood thus exists that a new, greener alternatives assessment identified based on NAMs will have equivocal risk rating compared to the compound of concern.

08.03 Microplastic: Standards, Big Data, and Analytical Methods in Research

08.03.01 ToMEx: Toxicity of Microplastics Explorer

L. Thornton Hampton, Southern California Coastal Water Research Project Authority / Toxicology; S.L. Coffin, University of California, Riverside / Environmental Toxicology; H.E. Lowman, University of Nevada, Reno / Aquatic Biogeochemistry & Environmental Data Science Department of Natural Resources and Environmental Science; E. Darrin, Southern California Coastal Water Research Project Authority / Toxicology; C.M. Rochman, University of California, Davis / Department of Ecology and Evolutionary Biology; A. Koelmans, Wageningen University / Aquatic Ecology and Water Quality Management Group; A.C. Mehinto, Southern California Coastal Water Research Project / Toxicology

Microplastics are an incredibly diverse contaminant suite, comprising a complex mixture of physical and chemical properties, many of which are known or suspected to influence toxicological outcomes. During the last decade, there has been a surge of research aimed at measuring and characterizing microplastic occurrence in the environment and biota, and in turn, studies aimed at describing the toxicological effects of microplastics have also dramatically increased. However, the diversity of microplastics is also reflected in toxicity data, as published studies comprise a diverse array of test organisms, particle types, experimental designs, and biological endpoints. This presents a difficult challenge in keeping pace with the most recent advancements in microplastic toxicity as well as identifying data that are appropriate for specific applications (e.g., hypothesis testing, risk assessment, etc.). Though individual studies may be informative on their own, there is currently no tool for efficiently and systematically summarizing, visualizing, and analyzing microplastic toxicity data. To address this need, the Toxicity of Microplastics Explorer (ToMEx) was created to illuminate what is known and unknown regarding the effects of microplastics on both human and ecological health. Here, over 200 peer-reviewed studies were data mined for information pertaining to five key categories: 1) Test Organisms 2) Experimental Design 3) Biological Effects 4) Particle Characteristics and 5) Quality Criteria. These underlying data are then summarized and may be visualized and analyzed using a web-based application powered by Rshiny. Users may use the application to filter and visualize the data by a number of variables pertaining to the previously mentioned categories and construct species sensitivity distributions using selected data. While ToMEx was originally designed for specific regulatory purposes for the state of California, its accessibility and interactive nature allow use by diverse audiences for a variety of research objectives.

08.03.02 Representative Subsampling Methods for the Chemical Identification of Microplastic Particles

H. De Frond, A.M. O'Brien, University of Toronto / Department of Ecology and Evolutionary Biology; C.M. Rochman, University of California, Davis / Department of Ecology and Evolutionary Biology

Chemically identifying microparticles is a critical endeavor of microplastics research, yet the high number of microplastics in samples and the time required to identify each makes this task costly. To save time, researchers often subsample particles for chemical identification – used to confirm particles as anthropogenic and/or plastic as well as identify material types. As no standard protocols exist for subsampling, methods vary widely. This leads to a lack of comparability between studies, and often no evidence to confirm that the subsample is representative. Adopting harmonized and representative subsampling practices will increase comparability among studies, and strengthen conclusions made from chemical identification results. Here we conduct "in silico" experiments to inform best practices when subsampling microparticles for chemical identification. Our research has two objectives: 1) to characterize the accuracy of anthropogenic particle and plastic identification via microscopy, 2) to accurately determine the diversity of polymer types in a sample. We used published datasets where 100% of microparticles counted were chemically characterized to test subsampling methods, and contrasted the cases where conclusions are needed for each sample individually versus for a sample set. We determine that overall, random particle selection provides the most representative subsampling method with the lowest effort (# of particles chemically identified). The minimum number of particles to be subsampled for chemical identification must be informed by the objective of your research – e.g., confirming particles are synthetic requires less effort than accurately reporting the diversity of material types. When selecting particles across multiple samples, subsampling is more representative when an equal number of particles are selected from each sample.

08.03.03 Potential High Throughput, Low-Cost Method Combining Polarized Light Microscopy and Nile Red Staining to Identify Microplastics

C. Knauss, University of Maryland Center for Environmental Science; J. Goodwin, Pelagic Dynamics; E. North, University of Maryland Center for Environmental Science

Identifying microplastics from the environment is time consuming and expensive because of the variety of plastic particles in the environment and the abundance of associated organic particles. To date, a reasonably accurate low-cost high-throughput method has not been developed, yet such a technique is needed to characterize microplastics in environmental samples. The objective of this study was to assess the ability of an automated image capture system to identify microplastics using polarized light in conjunction with Nile red staining. Most plastic polymers are crystalline structures and, when illuminated under cross-polarized light, show birefringence. In this study, particles (0.5-5 mm) of 15 known polymers, commonly found in the environment (e.g., polypropylene, low density- and high density-polyethylene, polyethylene terephthalate, PVC, nylon, polystyrene), were tested with the automated polarized light system, both with and without Nile red staining. A variety of sizes and colors were chosen to best represent environmental samples and microscope settings were optimized for microplastic birefringence. In addition, the polarized light technique with Nile red staining was tested with particles collected from the environment and are being validated with attenuated total reflection (ATR)-FTIR. Results indicate that 9 of 15 microplastic polymers are birefringent, 10 of 15 are fluorescent with Nile red, and 14 of 15 are either birefringent or fluorescent with Nile red. Polarized light microscopy identified polypropylene and low density- and high density-polyethylene while Nile red staining could not. Nile red staining did not affect birefringence, hence 93% of the 15 plastic types could be identified as plastics with the combined techniques. Further results testing this combination, verified by ATR-FTIR, with environmental particles will be presented and discussed. The combination of these two techniques provides a low-cost and potentially high throughput method to identify and quantify microplastics in environmental samples.

08.03.04 Leveraging Big Data to Predict Microplastics Toxicity for Aquatic Organisms

S.L. Coffin, University of California, Riverside / Environmental Toxicology; L. Thornton Hampton, Southern California Coastal Water Research Project Authority / Toxicology; A. Koelmans, Wageningen University / Aquatic Ecology and Water Quality Management Group; W. Cowger, University of California, Riverside / Environmental Science; M. Kooi, Wageningen University & Research / Aquatic Ecology and Water Quality Management Group

Microplastics are one of the most diverse and complicated classes of environmental contaminants. The Microplastics contaminant suite includes particles with thousands of unique polymer types, ranging in size from nanometers to millimeters, a continuum of shapes, densities, known and unknown associated contaminants, pathogens, and more. Despite abundant experiments testing the toxicity of microplastics on a plethora of unique species and endpoints, many fundamental questions remain regarding how microplastics affect organisms and at what concentrations. Such questions include: what drives toxicity, is size important, and which dose metric best predicts toxicity (mass, particle count, volume, etc.)? To illuminate what's known and unknown regarding the effects of microplastics in aquatic ecosystems, we mined data from 167 peer-reviewed studies and tested several models to predict toxicity. A training database with more than 6,000 monodisperse microplastics toxicity measurements was used to predict the toxicity of an environmentally realistic polydisperse mixture of microplastics. While a random forest model most accurately predicted toxicity, a binomial logistic regression model was more generalizable and interpretable, while still providing accurate predictions. Feature importance analysis revealed particle size and polymer type as important predictors of toxicity for a given endpoint/species, with particle shape being significantly less important. Additionally, stepwise multiple regression analysis revealed total particle surface area per volume as the best predictive exposure concentration metric compared with volume normalized total particle count, total mass, or total particle volume. This modeling approach is a novel method to identify key drivers of toxicity and provides insights into how microplastics affect aquatic organisms, informing future risk assessment frameworks for microplastics and other complex contaminant suites.

08.03.05 Trash Taxonomy Tool: Harmonizing Classification Systems Used to Describe Trash and Microplastics in the Environment

H. Hapich, W. Cowger, A.B. Gray, University of California, Riverside / Environmental Science

There has been rising concern about the threat of plastic pollution to environmental and human health. Many groups around the world now monitor trash and microplastics in the environment. Harmonizing data from these surveys remains unsuccessful mainly because there is no standard classification system or framework for relating between systems, which inhibits the advancement of regional to global synthesis of trash and microplastics. We assessed 68 current surveys that describe trash and microplastic categories and found that item classes were on average 20.8% comparable, and 29.9% comparable for material classes; around half of the survey sheets were not comparable with one another at all. With such low comparability, the Trash Taxonomy Tool attempts to tackle this issue by harmonizing existing survey classes using a series of relational and hierarchical tables and proposing a new standardized naming system informed by existing ones. The datasets and tools are hosted on an open-access website (https://wincowger.shinyapps.io/TrashTaxonomy/), allowing users to insert their data into the hierarchical system, as well as download the relational tables directly. We are expanding this tool to also improve the comparability and usability of data between microplastic studies. By increasing preexisting and new data usability, we can better support integrative trash and microplastic investigations across broader spatio-temporal scales, and expedite management and policy decisions that rely on such survey data.

08.03.06 Application of a Hybrid Fusion Classification Process for Identification of Microplastics Based on FTIR Spectroscopy

B. Chabuka, Idaho State University / Department of Chemistry

Consistent accurate identification of microplastic polymer composition is vital for understanding the impact of microplastic pollution in the environment. Fourier transform infrared (FTIR) spectroscopy is becoming common place for identifying microplastics. Conventional spectral identification is based on library searching, a process that utilizes a search algorithm against digital databases containing single spectra of pristine reference plastics. Several conditions on environmental microplastic particles such as weathering, additives, and residues cause spectral alterations relative to pure reference library spectra. Thus, library searching is vulnerable to misidentification of microplastic samples. While a classification process (classifier) based on a collection of spectra can alleviate the misidentification problem, optimization of each classifier (tuning parameter) is required. Additionally, erratic results relative to the particular optimized tuning parameter can occur when the microplastic samples originate from new environmental or biological conditions than those defining the class. Presented in this study is a process that utilizes spectroscopic measurements in a hybrid fusion algorithm that depending on the user preference, simultaneously combines high-level fusion with low and mid-level fusion based on an ensemble of non-optimized classifiers to assign microplastic samples into specific plastic categories (classes). The approach is demonstrated with seventeen classifiers using FTIR for binary classification of polyethylene terephthalate (PET) and high-density polyethylene (HDPE) microplastic samples from environmental sources. Other microplastic types are evaluated for non-class PET and HDPE membership. Results show that high accuracy, sensitivity, and specificity are obtained thereby reducing the risk of misidentifying microplastics.

08.03.07 Documenting Derelict Fishing Gear in Hawaii Using Analytical Methods: Creation and Use of Large Datasets

R. Corniuk, Hawaii Pacific University; A. McWhirter, G. Boyd, Hawaii Pacific University Center for Marine Debris Research; H.W. Lynch, Makanakai Marine Services; S. Royer, Hawaii Pacific University Center for Marine Debris Research; J. Lynch, National Institute of Standards and Technology

Derelict fishing gear (DFG) comprises a significant amount of plastic pollution in the ocean, causing both environmental and economic losses. Building evidence and compiling data on DFG is crucial to holistically develop solutions to reduce the amount and impacts of DFG. The goal of this study is to document the gear type, construction and chemical composition of DFG bundles using a standardized method to ultimately source it back to its original fishery. To date, 127 DFG bundles have been removed across the Main and Northwestern Hawaiian Islands and the North Pacific Gyre, totaling 21 metric tons. Within a DFG bundle, each individual gear type, such as nets, lines, floats and hard plastic mesh, is thoroughly dissected and documented in a database including over 70 gear configuration metrics per sample (e.g. color, rope diameter, net mesh stretch size). Evidence such as foreign languages, manufacture date, and unique objects associated with fishing gear are also being investigated as it could help trace the DFG to its original source. In addition, Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy is used for polymer composition and Differential Scanning Calorimetry analysis is conducted to 1) differentiate density classes of polyethylene (high, linear low, and low density) and 2) identify the percentages of polyethylene and polypropylene for polymer blends. As a result, after lab analysis has been completed on the 127 bundles, there will be over 2 million cell entries. The information housed in this database is crucial for getting a better understanding of the complexities of various fishing gears and to aid in identifying the sources with the ultimate goal of developing DFG preventive measures that will reduce the total amount of fishing gear entering the marine environment and its eventual fragmentation into microplastics.

08.03.08 Quantification of Microplastics Using Microwave Assisted Extraction and Pyrolysis-Gas Chromatography/Mass Spectrometry Py-Gc/Ms

L. Hermabessiere, University of Toronto / Ecology and Evolutionnary Biology; C.M. Rochman, University of California, Davis / Department of Ecology and Evolutionary Biology

The global contaminant microplastics (MP) is very diverse in terms of polymer, shape and size. This complexity leads to challenges for quantifying them in the environment. Historically, MPs have been quantified and chemically identified using spectroscopic techniques, Fourier-transform infrared (FTIR) or Raman spectroscopy, with microscopy. This process is labor intensive and time consuming. Recently, Py-GC/MS was proposed to rapidly quantify and identify MPs. In this study, a new extraction method using Microwave-Assisted Extraction (MAE) combined with Py-GC/MS was developed to extract and quantify a wide range of plastic polymers and validated using different environmental matrices. This new extraction method had good recoveries (92.94% - 119.74%) and was able to extract polyethylene (PE), polystyrene (PS), polypropylene (PP), poly(methyl-methacrylate) (PMMA), polyvinylchloride (PVC) and polycarbonate (PC) in dichloromethane. Repeatability was appropriate when measured both within one day and across several days, with coefficients of variation among subsamples below 25% across all polymers. The limit of detection (LOD) and limit of quantification (LOQ) of the method ranges from 0.002 to 0.18 µg and 1.16 to 5.85 µg, respectively. A spike recovery was included in the method validation and consisted of all polymers added to various environmental matrices. Recoveries for all polymers in clean water, dirty water, shrimp and salmon fillet samples were between 85-111%, 87-138%, 81-122% and 50-151%, respectively. In addition, the method was tested using bottled water and wild mussels for proof-of-concept. For bottled water, PP, PS and PC were all detected below the LOQ. For wild mussels, PE and PVC were detected and quantified in mussels, and PC and PP were detected below the LOQ. Here, a method combining MAE and Py-GC/MS is introduced as a tool for mass quantification of microplastics. This method can be used as a stand-alone, or as a complementary method to spectroscopic techniques.

08.03.09 Automated Hyperspectral Imaging and Chemical Identification of Environmental Microplastic in an Urban Watershed

S. Patankar, Ocean Wise Conservation Association / Research; S. Wang, A. Parizi, Oceanwise / PlasticLab; P.S. Ross, Vancouver Aquarium / Ocean Pollution Research Program; A. Posacka, Ocean Wise Conservation Association / Coastal Ocean Research Institute

Environmental prevalence of microplastics is an emerging issue of global concern. The complexity of sources and fate of these novel contaminants continues to challenge an efficient and robust chemical identification, and effective mitigation strategies. Urban pathways, such as municipal drainage systems, wastewater treatment plants and rivers, play a major role in contributing microplastics to the environment. The relatively high microparticle density in samples from urban samples complicates accurate chemical information of microplastics and other constituent particles. Here we present an automated chemical identification method for urban matrices based on reflectance-Fourier Transform Infrared Spectroscopy (FTIR) hyperspectral imaging, integrated with structured data storage and quality assurance/quality control analysis pipeline protocols. Our data analysis workflow was developed as part of extensive urban watershed studies, and incorporates rapid processing of large and complex infrared spectral datasets, along with extraction of quality chemical identity of environmental particles.

08.03.10 Customization and Standardization: Supporting Local Data Collection and Tracking Urban Litter with Marine Debris Tracker

K. Youngblood, University of Georgia / College of Engineering; J. Jambeck, University of Georgia

Participatory sensing of plastic pollution on a global scale using citizen science poses unique challenges of data standardization. Marine Debris Tracker is a free mobile application used by researchers, on-profit organizations, and citizen scientists around the world to collect open access geospatial data on plastic pollution. With over 4 million items logged to date in 92 different countries, Debris Tracker has grown largely through supporting customized data collection lists adapted to language, dialect, context, and specific research questions or interests of local organizations. Debris Tracker also supports multiple types of data collection – uploading highly accurate geospatial data for individual litter items through the app in real time and uploading aggregated datasets from cleanups manually online. Balancing ease of data collection and accuracy is especially key for efforts relying on citizen scientists to collect data. To adapt to local contexts, Debris Tracker provides customizable item names and categories, which are tied to a master item characterization schema and corresponding material types. Although customization has been a large incentive for organizations to share their data with Debris Tracker's open database, standardization is necessary to allow comparability between locations. This characterization list was developed based on NOAA's Shoreline Survey Methodology and expanded organically as new items emerged in local data collection schemas. Path logging – recording the path followed by trackers as a metric of where debris is not – allows an additional degree of backend standardization. Much citizen science data collected on plastic pollution has occurred in environmental sinks, presenting challenges in bias of location selection for the most littered sites, among others. Urban litter is a new frontier of plastic pollution data collection that allows data capture close to the source of where litter is generated due to human activities. Recently developed citizen science data collection methods for urban litter as part of the Mississippi River Plastic Pollution Initiative (https://www.unep.org/mississippi) were adapted from methods piloted along the Ganges River as part of National Geographic's Sea to Source Expedition. Piloted in three cities over a one-month data collection period, over 75,000 items were recorded as part of the initiative.

08.03.11 Open Specy: An Open-Source Online Tool for Raman and FTIR Spectral Analysis of Microplastics and Beyond

W. Cowger, A.B. Gray, University of California Riverside / Environmental Science

Spectroscopy is an essential tool for microplastic research. Spectral analysis is used to characterize polymer types, mixtures, and weathering, and provide an independent evaluation of particles identified through taxonomic approaches. Microplastic spectral analysis is a rapidly developing field with a diversity of methods, which makes comparability and reproducibility across studies challenging. Spectral analysis software and libraries can cost thousands of dollars, yet often do not have reference materials that are relevant to microplastic research. Several open access spectral software and reference databases have been developed that include weathered and non-virgin polymer materials absent from many commercial libraries. Open Specy is an online (www.openspecy.org), open-access spectral analysis tool that includes many of these reference databases, and encourages users to submit reference spectra to keep up with the ever-growing diversity of plastic materials in the environment. Open Specy allows researchers to freely and accurately process and identify FTIR and Raman spectra of environmental plastic pollution.

08.03.12 Screening for Plastic Debris in Agricultural Soil - an Open Data Approach

Z. Steinmetz, P. Löffler, K. Muñoz, G.E. Schaumann, University of Koblenz-Landau / iES Landau, Institute for Environmental Sciences

The use of agricultural plastic covers offers increased yields and an improved crop quality. The most applied materials are polyethylene (PE) films and polypropylene (PP) fleeces. Although made to last for up to five years, parts of these covers are suspected of breaking down into smaller debris due to physical stress and may thereby contribute to soil contamination with microplastics. To scrutinize this, we randomly sampled 240 topsoil cores (0–5 cm) from eight fields covered with fleece, perforated foil, and/or plastic mulch. Samples from the field periphery (50 m perimeter) served as reference. Visual plastic debris >2 mm were analyzed by FTIR–ATR and identified using the Open Specy library. Smaller plastic debris were dispersed from 50 g of fine soil (≤2 mm) using sodium hexametaphosphate solution and density-separated with saturated NaCl solution. The collected PE, PP, and polystyrene (PS) debris were selectively dissolved in a 1:1-mixture of 1,2,4-trichlorobenzene and *p*-xylene at 150 °C, quantified by pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS), and evaluated with OpenChrom and R statistical software (all data will be made publicly available upon submission of the manuscript preprint). We counted eight PE and PS fragments >2 mm in two out of eight fields. By contrast, Py-GC/MS analysis revealed PE, PP, and PS contents >1 $\mu g\ g^{-1}$in seven fields (18% of all samples). PE and PP levels of up to 7 $\mu g\ g^{-1}$were probably associated with the use of thinner and less durable perforated films or fleeces. This was most pronounced at field edges where agricultural covers are typically turned and weighted down with soil or sandbags. On one site, we observed expanded PS particles >2 mm that concurred with elevated PS levels (8–19 $\mu g\ g^{-1}$) in the fine soil; but their source remained unresolved. In addition, the extent to which increased PE contents of up to 7 $\mu g\ g^{-1}$in the field periphery may be attributed to wind drift of plastic covers or other, external sources needs to be addressed in future studies.

08.03.13 Microplastic Contamination in Tiger Sharks (Galeocerdo cuvier) from the Atlantic Coast of the United States

K. Munno, University of Toronto / Ecology and Evolutionary Biology; L. Hoopes, Georgia Aquarium; K. Lyons, Georgia Aquarium / Research and Conservation; C.M. Rochman, University of California, Davis / Department of Ecology and Evolutionary Biology

Macroplastic and mesoplastic ingestion has been observed in several shark and other elasmobranch species. Few studies have documented microplastics (< 1 mm) in shark gastrointestinal (GI) tracts. Tiger sharks (*Galeocerdo cuvier*) are apex predators and consume a wide array of food items relative to other species. As more generalist feeders, tiger sharks may ingest microplastics and other plastics. Overall, we examined the GI tracts of seven individuals captured on the Atlantic coast of the United States. Shark GI tracts were chemically digested using potassium hydroxide (200 g/L) and density separated in a calcium chloride (1.4 g/mL) solution before quantifying and categorizing suspected anthropogenic particles (>45 µm) by colour and morphology. All sharks contained anthropogenic particles. Across all seven sharks, a total of 3152 anthropogenic particles were found, with up to 1603 anthropogenic particles in a single shark. A subset of suspected anthropogenic particles (14%) were chemically identified using Raman spectroscopy to determine the range of polymer types observed and confirm anthropogenic origin. We found diverse microparticle morphologies, with fragments (57%) and fibers (41%) most frequently observed. Overall, ≥95% of particles analyzed via spectroscopy were confirmed anthropogenic, with 49% confirmed as microplastics. Of the microplastics, polypropylene (32%) and polyethylene (10%) were the most common polymer. The high occurrence and abundance of anthropogenic particle contamination in tiger sharks is likely due to their generalist feeding strategy and trophic position. The GI tracts were paired with blood samples taken from the same sharks to characterize any translocation of particles using pyrolysis gas chromatography – mass spectrometry. All data gathered to date will be presented. Combined, our research will help us understand the fate of micro- and nano- plastics in large fish.

08.03.14 Polymer Kit 1.0: Usefulness of Polymer Reference Materials in Marine Debris Analyses

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The plastic pollution research community has been hindered by the lack of well characterized polymer standards in the microplastics size range (< 5 mm). Microplastic standard material options have previously been limited and costly. In 2020, in collaboration with the American Chemistry Council and their member companies, the Hawaii Pacific University Center for Marine Debris Research (CMDR) produced Polymer Kit 1.0, an affordable and convenient set of microplastic polymer standards with the aim of harmonizing plastic pollution research. Polymer Kit 1.0 contains 22 diverse plastic materials typically found in the environment and a USB data drive with a written report of CMDR's preliminary analysis, attenuated total reflectance Fourier-transform infrared spectroscopy (ATR FTIR) spectra, and differential scanning calorimetry (DSC) curves for each material. The kit also includes an invitation to the Polymer Kit Network, a Google Group where kit users can connect. To date, 62 Polymer Kits have been distributed to researchers in academia, industry, and government across 11 countries worldwide. Polymer Kit 1.0 has been used for a wide range of research needs, including building in-house FTIR and Raman libraries, performing pyrolysis gas chromatography mass spectrometry (Py-GCMS) calibrations, producing smaller microplastics and/or nanoplastics, testing biological impacts, and executing degradation, transport and fate experiments. Details regarding kit components, the accompanying data, and several case studies showing how the kit is being utilized across different laboratories will be presented. Finally, CMDR recognizes the diversity of needs within the microplastic research community and hopes to gain feedback from the participants in this session regarding what people want to see included if a Polymer Kit 2.0 is developed.

08.03.15 Fast and Reproducible Analysis of Infrared and Raman Spectra of Microplastics Using Machine Learning

L. Wander, B. Hufnagl, Purency GmbH; H. Lohninger, Vienna University of Technology / Institute of Chemical Technologies and Analytics; E. Kanaki, F. Fischer, D. Fischer, Leibniz-Institut f. Polymerforschung Dresden; H. Martirosyan, M.G. Löder, University of Bayreuth / Animal Ecology I; C. Laforsch, University of Bayreuth

Quantifying microplastics from environmental samples can be challenging and many different methods have been developed to address this problem. Despite a lack of uniformity in the field of microplastic analysis, some approaches have proven successful and are widely used. One prominent example is the use of vibrational spectroscopy for the identification of microplastics. Spectroscopy is usually part of a multi-step analytical procedure consisting of sampling, sample preparation, measurement, and the analysis of the data. Due to the complexity of this multi-step process, the comparability of results among different laboratories is still a problem and a driving forces behind current harmonization efforts. However, many problems associated with the last step, analyzing the spectra, can be solved by model based and automated analysis routines. Some of the difficulties interpreting the spectra manually, arise from the many physical and chemical effects which can alter the appearance of spectra. This includes the scattering of light, varying thickness of the particles partially causing total absorption, fluorescence, and many more. Together they increase human bias introduced during the interpretation of the spectra and make the analysis a time-consuming task. Here, we demonstrate the benefits of model-based machine learning using random decision forests (RDF) to significantly simplify the process. Microscopic chemical images obtained with focal plane array detectors using infrared spectroscopy in transmission mode were chosen to show the great potential of the approach. Images comprised of millions of spectra can be analyzed within only minutes identifying the 20 most common polymer types. The analysis works reliably for different environmental matrices such as air, water and soil and is complemented by an automated particle size characterization. Spectral analysis by RDF classifiers is not limited to infrared data and has also been successfully transferred to Raman spectra. In a proof of concept, we trained a model to identify five common polymer types and evaluated its performance using spectra from different laboratories. One of the main advantages of the approach is the lower false positive rate and thus lower effort for manual post-processing of the results compared to the data analysis routine used so far.

08.04 Model Ecosystem Scale Studies

08.04.01 RNA Metabarcoding of Bulk Zooplankton Samples to Assess Anthropogenic and Environmental Influences

P. Ankley, Y. Xie, University of Saskatchewan / Toxicology Centre; S. Havens, IISD Experimental Lakes Area (IISD-ELA) / Chemistry; L. Peters, University of Manitoba / Department of Chemistry; J. Rodriguez Gil, IISD Experimental Lakes Area (IISD-ELA) / Department of Biology; L. Timlick, M. Paterson, IISD - Experimental Lakes Area; G. Tomy, Fisheries and Oceans Canada / Department of Chemistry/COGRAD Laboratory; J.P. Giesy, University of Saskatchewan / Department of Veterinary Biomedical Sciences and Toxicology Centre; V. Palace, IISD Experimental Lakes Area (IISD-ELA)

Community DNA metabarcoding provides a rapid and high-throughput method for characterizing zooplankton responses to environmental stressors. DNA metabarcoding, however, cannot distinguish live organisms from carcasses, while RNA metabarcoding could allow for detection of community vitality and more transient responses to stressors. A freshwater mesocosm study was conducted to assess efficacies of various alternative secondary remediation practices for a simulated oil-spill, with zooplankton metabarcoding being applied for ecotoxicological assessment. Normalized vitality was also assessed, namely RNA: DNA metabarcoding ratio, for testing the ability to decipher community response to treatments. Metabarcoding indicated *Cyclops sp.* and *Keratella sp.* dominated the zooplankton community. DNA metabarcoding detected decreases in alpha diversity in enclosures treated with a shoreline cleaning agent at 38 days post-spill relative to reference and 3 days pre-spill, with RNA indicating no change. Normalized vitality was able to decipher changes in community due to seasonal variations and detect differences between zooplankton phyla Arthropoda and Rotifera. Overall, metabarcoding can serve as an efficient and descriptive approach for characterizing community response to stressors and can be of use to spill responders and decision-makers for ecotoxicological assessments. There are still limitations with this method that will need to be continuously addressed with benchmarking and development, including curated databases and optimized primers for target communities.

08.04.02 Ecosystem Scale Studies Have a Disproportionate Influence on Environmental Policy and Regulations

J. Rodriguez Gil, IISD Experimental Lakes Area (IISD-ELA) / Department of Biology; V. Palace, IISD Experimental Lakes Area (IISD-ELA)

Environmental toxicologists are increasingly making use of modelling approaches, genomic techniques, and alternatives to tissue and whole animal testing. These advancements are overwhelmingly positive from a cultural perspective and offer the advantages of replicability and repeatability. Alternatively, manipulation studies at the ecosystem scale can examine the effect of changing key variables to examine effects within the same context as other ecosystem scale processes. For the past several decades the IISD-Experimental Lakes Area has conducted large ecosystem-scale studies to investigating the fate, effects, and remediation strategies for contaminants in freshwater environments. Here we present evidence that results from these, and other similar approaches, have had a disproportionate influence on environmental policy and regulations. We also provide examples of how ecosystem scale studies can be useful for calibrating studies at organism, tissue, and individual levels to ensure that current and future ecotoxicological models can properly predict processes at higher levels of organization.

08.04.03 Effects of Venlafaxine on a Boreal Lake Ecosystem Using Mesocosms

H. Jovanovic, L. Timlick, University of Manitoba; V. Palace, IISD Experimental Lakes Area (IISD-ELA); M.L. Hanson, University of Manitoba / Department of Environment and Geography; J. Rodriguez Gil, IISD Experimental Lakes Area (IISD-ELA) / Department of Biology

Venlafaxine is a commonly prescribed antidepressant drug, which can enter freshwater ecosystems via wastewater effluent and has been detected in surface waters at >2.0 µg/L. Despite its pseudo-persistence and expected chronic exposure, studies on the environmental fate and effects on freshwater ecosystems are lacking. A 10-week exposure using in-lake mesocosms was conducted at the International Institute for Sustainable Development – Experimental Lakes Area (IISD-ELA) to assess the fate and effects of venlafaxine in an ecological context (individual-community-level). Mesocosms (n=10, 2m diameter, 1.5m deep) were deployed in an experimental lake (Lake 239) and spiked with venlafaxine at varying concentrations in a regression design ranging from 0 µg/L (control; in triplicate) to 100 µg/L (n=1 per treatment). Each enclosure was stocked with 5 finescale dace (*Chrosomus neogaeus*) prior to spiking. Exposure levels were maintained at the target concentration by weekly re-spiking weekly over the study period, as the half-life of venlafaxine was estimated at approximately 5 days. Periphyton, phytoplankton, zooplankton, benthic and emergent invertebrate biomass, community composition, and growth metrics (length and weight) of adult finescale dace were monitored. Bioaccumulation potential in fish and periphyton was also be assessed. In-lab, standard 7-day embryo-larval exposures were conducted using wild collected fathead minnow (*Pimephales promelas*) eggs, assessing percent hatch, mortality, and developmental malformations. Behavioural testing with fathead minnow larvae was conducted to estimate predator avoidance behaviour. By understanding the environmental fate, direct and indirect ecological effects, and bioaccumulation potential, we will be able to estimate the risk of venlafaxine to the environment as it is currently being discharged in wastewater treatment plant effluent.

08.04.04 Evaluating the Interactive Effects of Diluted Bitumen, Crude Oil and Ultraviolet (UV) Radiation to Hyalella azteca

S.M. Michaleski, University of Manitoba / Biological Sciences; A.J. Bartlett, Environment and Climate Change Canada / Water Science and Technology Directorate; L. Timlick, IISD - Experimental Lakes Area; M.G. Barron, U.S. Environmental Protection Agency / Office of Research and Development; L.E. Peters, University of Manitoba / Department of Chemistry; K. Jeffries, University of Manitoba / Biological Sciences; V. Palace, IISD Experimental Lakes Area (IISD-ELA)

Potential environmental impacts of oil spills are a concern for the Canadian public and the oil industry. Crude oils and diluted bitumen (bitumen thinned with natural gas condensates so that the product can be transported in pipelines) are major products of the oil sands region. The rate of crude oil and diluted bitumen spills has declined over the past decade, but the risk of spills into aquatic environments is still a large concern, especially in freshwaters where little is known about the interaction of oil in the environment. Previous studies have evaluated the toxicity of crude oils and diluted bitumen, but many overlook the potential for photo-enhanced toxicity of oil constituents in environments with UV exposure. To examine the interactive effects of these oils and UV radiation, *Hyalella azteca* were exposed to water accommodated fractions of crude oil and diluted bitumen from shoreline enclosures of the Freshwater Oil Spill Remediation Study (FOReSt) at the IISD-Experimental Lakes Area, under low (10%) and high (90%) UV exposure. UV was also evaluated in small boreal lakes at the IISD-Experimental Lakes Area to model UV penetration and estimate areas of high risk for *Hyalella azteca* in natural lake settings.

08.04.05 Synthesis: A Framework for Predicting the Dark Side of Ecological Subsidies

J.M. Kraus, U.S. Geological Survey / Columbia Environmental Research Center; J. Wesner, University of South Dakota / Biology; D. Walters, U.S. Geological Survey / Columbia Environmental Research Center

Chemical contaminants influence ecological systems and patterns of species distribution at a global scale. Thus, understanding and managing the extent and magnitude of the effects of contaminants on ecosystems will continue to be one of the major challenges of this century. Here we review heuristic models useful for predicting the effects of chemical contaminants on food webs at the land-water interface. These models allow us to generalize empirical findings to new environments; more efficiently utilize time and monetary resources by providing information about what, when, and where to measure effects and to derive and test hypotheses that separate causal versus correlative agents of ecological change. We focus on effects of contaminants on production of animals with complex life histories, use and quality of terrestrial detritus to aquatic ecosystems, and the flux of contaminants through food webs both within and across ecosystem boundaries. We then construct a synthetic framework using conceptual control (a.k.a. programmatic) and food web models that pull together many of the factors and processes reviewed, including landscape, chemical/ecotoxicological, and ecological factors. This framework helps to identify (1) patterns of contaminant effects on ecosystems, and (2) data gaps and areas for future research.

08.05 Socio-Ecological Systems and Their Responses to Man-made Pollution Disasters

08.05.01 Redefining Critical Infrastructure for Community-Based Risk, Vulnerability and Resilience Assessment

D. Henshel, Indiana University / Public and Environmental Affairs; J. Ashby, V. Brown, G. Depper Coral, Indiana University - Bloomington

Predictions of future combined impacts of climate change and other major stressors on a socioecological system need to be based on assessment of the 3 main components of such systems: populations, structures, and infrastructures. Within the USA, infrastructures of greatest governmental concern are the critical infrastructure sectors, the proper functioning of which are considered essential to societal function. In order to better assess community-level vulnerability, risk and resilience to landscape scale stressors, the critical infrastructure sectors need to match the perceptions and needs of communities and individuals within communities. We have recharacterized the critical infrastructure sectors from a community-focused perspective in order to develop assessment and measurement metrics for climate change stressor-related impacts on socioecological systems. We simplified the 16 national critical infrastructure sectors as defined by the Department of Homeland Security (DHS) based on community perspectives, and grouped them into physical, social, and natural systems. For example, while there are 3 national Critical Infrastructure sectors that provide energy (Energy, Nuclear, Dams), the Community-Focused Critical Infrastructure Framework has only one Energy sector as the end user only cares about having energy available. Housing and education are not regulated at the federal level, so the DHS Critical Infrastructure sectors do not include either housing nor education. To communities, however, housing and education are critical infrastructures and so each is a sector in the Community-Focused Critical Infrastructure Framework. This re-evaluation and re-framing of the critical infrastructure sectors is essential for creating assessment categories, and thus assessment and measurement metrics, for community level risk, vulnerability (and resilience) impacts on the socioecological systems that are being increasingly adversely affected by global stressors like climate change. We propose this new Community-Focused Critical Infrastructure Framework to stimulate discussion and consideration of potential missing sectors or changes in the framework details.

08.05.02 Nature-Based Solutions to Improve Resilience to Disasters in Environmental Justice Communities

G. Newman, Texas A&M University / Landscape Architecture and Urban Planning; G.P. Sansom, L. Fawkes, Texas A&M University School of Public Health / Environmental and Occupational Health Sciences; R. Zhu, Texas A&M University / Landscape Architecture and Urban Planning; J. Horney, University of Delaware / Epidemiology

Background: In urban environmental justice (EJ) neighborhoods along the U.S. Atlantic and Gulf coasts, residents live in at the nexus of interlinked social, economic, environmental, and public health issues. Many such communities face risks from exposure to flooding associated with hurricanes and tropical storms, stormwater runoff, sewage overflows, and hazardous substance mobilization due to close proximity to industrial land uses. Vacant homes, abandoned parcels, and high pollution loads increase potential for floodwater to transport contaminants and increase opportunity for contact. High social vulnerabilities in these neighborhoods mean poverty, high rates of chronic disease, and poor quality housing and transportation exacerbate health impacts. Methods: First, neighborhood transportation hierarchy, current land use configurations, inundated area in 100-and 500-year flood plains, point and nonpoint source pollutants, sea level rise scenarios, and other factors are spatially assessed and visualized in a site inventory. Systematic community engagement is used to collect resident input, prioritize local knowledge and concerns, and create bidirectional communication flows. Master plans and site-specific plans including green infrastructure prevention and intervention strategies are developed and refined in an iterative process with residents and stakeholders. Finally, plan performance in reducing exposure to potential environmental health risks are quantified and assessed using performance models and calculators. Results: A master plan for Sunnyside, Texas, an EJ neighborhood in Houston, includes bioswales, rain gardens, and detention ponds that reduce resident exposure to environmental contaminants and increase resilience to flooding. Specifically, green infrastructure reduces runoff by 37% and reduces 10 of 13 common urban pollutants (e.g. nitrogen, phosphorus, suspended solids). Similarly, plans for the Northeast neighborhood of Wilmington, Delaware reduce runoff by 19.1% while reducing nonpoint source pollution by 3.7 – 77.2%, depending on the contaminant. Discussion: Community-engaged plans focused on using green infrastructure to remediate and reduce future environmental health risks from chronic pollution and acute disasters and emergencies can demonstrate significant reductions in exposure, improve resilience to future events, and provide financial return on investment. However, while nature-based solutions are typically cheaper than engineered, the scope and scale of implementation can be a barrier in EJ communities and implementation can increase property value. Therefore, disinvestment is common and green gentrification can be a concerns.

08.05.03 A Systems-based, Whole Community Approach to Preparing for and Responding to Man-Made Disasters to Reduce Risk for Human and Ecologic Harm

S. Croisant, The University of Texas Medical Branch / Preventive Medicine and Population Health; M. Mastrangelo, The University of Texas Medical Branch / Facilities Risk Management; L. Hallberg, The University of Texas Medical Branch / Sealy Center for Environmental Health and Medicine; J. Prochaska, The University of Texas Medical Branch / Preventive Medicine and Population Health; C. Singleton, The University of Texas Medical Branch / Sealy Center for Environmental Health and Medicine; K. Bohn, The University of Texas Medical Branch / Institute for Translational Sciences; D. Reilly, National Oceanic and Atmospheric Administration / National Weather Service; T. Munoz, City of Texas City / Emergency Management/Homeland Security; C. Elferink, The University of Texas Medical Branch / Pharmacology and Toxicology

We embrace an Integrated Socio-Ecological System for the Gulf of Mexico that utilizes evidence-based approaches to inform management of natural resources, with a goal of delivering sustainable levels of ecosystem services. As coastal populations grow, demand for access to benefits—and risk of resource depletion or damage—is increasing. The Socio-Ecological System implies that sustaining ecosystem services is contingent upon understanding and protecting the environments from which they are derived. Thus, improving capacity to prepare for, respond to, and recover from disastrous environmental events is vital. Numerous natural and man-made disasters have repeatedly tested coastal communities. We propose adopting a *Whole Community Approach* to community resilience, which brings to bear the full capacity of the private and nonprofit sectors to prevent, protect against, mitigate, respond to, and recover from multiple threats or hazards effectively. We are engaging diverse stakeholders from science, medicine, community groups, industry, local and regional emergency response officials, and policymakers. One of the most dangerous potential disasters in this region is posed by Hydrogen Fluoride (HF), used in large volumes in gasoline refining. If released into the atmosphere, HF reacts with moisture in the air to form a highly toxic, heavier-than-air vapor cloud. One industrial plant in the region has over 1 M pounds of HF in circulation, and a recent Consequence Analysis revealed that a worst-case release would result in a 25-mile plume with ~640 K residents affected. However, even for a much smaller incident with 2,000 exposures, current medical response plans and resources would be inadequate. Major accidents have occurred in regional HF facilities, including a significant incident in 2016 when 16 workers were hospitalized and another as recent as May 2021. UTMB is engaged in developing a framework to improve medical and community response plans—and importantly, community preparedness and resiliency—through a series of stakeholder-engaged projects including:

risk assessments to identify and inform preparedness efforts for region-specific chemical threats; modeling of likely release scenarios to drive policy decisions and response actions; tailoring of CHEMPACK contents based on risk assessment, development of integrated Whole-Community exercise programs to assess readiness, capabilities, and capacity, and creation and utilization of new risk communications models.

08.05.04 Networks Analysis of Social Survey Data Following a Pollution Disaster to Identify Key Societal Socio-Ecological Concern Areas

D. Hala, Texas A&M University, Galveston / Marine Biology; A. Ross, Texas A&M University, Galveston / Department of Marine and Coastal Environmental Science

Man-made or anthropogenic pollution spills or disasters appear to be an ubiquitous and chronic stressor in afflicted ecosystems. While a monetary measure of pollution disturbances on built infrastructures and ecosystem services can be relatively easily observed and quantified, it is more difficult to estimate societal concerns. Furthermore, the adverse effects of such pollution disasters on interacting (and inter-dependent) socio-ecological systems can exacerbate societal concerns and erode community resilience. Therefore, there is a need for methods that can incorporate and integrate across a diversity of societal concern areas in order to identify co-occuring concern categories, discern neglected categories, and identify information or knowledge gaps. In this presentation, results from the implementation of a networks analysis approach to study social survey data will be presented. Specifically, the analysis of social surveys performed after the petrochemical fire and spill at Deer Park (March 2019, Houston, TX) will be presented. The results presented aim to provide a roadmap for targeted intervention strategies that may alleviate community concerns and address information gaps.

08.05.05 Investigating Impacts of the Pandemic on Plastic Pollution Using Citizen Science Databases

B. Ertel, J.E. Weinstein, The Citadel / Department of Biology

The COVID-19 pandemic has affected global human health in profound ways and resulted in a marked increase in the use of personal protective equipment (PPE) such as face masks and gloves. Both disposable and reusable PPE items are typically made of plastic materials and may pollute local environments if improperly disposed. Plastic pollution is one of many ways our anthropogenic activities negatively affect environmental health, but litter cleanups provide opportunities to promote local ecosystem resilience. The objective of this study was to document trends in PPE litter abundance reported by community volunteers in both national (U.S.) and local South Carolina litter cleanups. By utilizing public litter databases maintained by the Ocean Conservancy (TIDES) and the South Carolina Aquarium (Litter-free Digital Journal), we tracked temporal trends in the number of citizen science volunteers, number of cleanups, plastic litter composition, and PPE abundance throughout the pandemic. Compared to historic data from 2016-2019, in 2020 there were fewer community volunteers but more total cleanups. This suggests that volunteers continued to collect and remove litter but did so in smaller groups to allow for social distancing. The number of PPE items collected in U.S. cleanups (n= 23,599) and SC cleanups (n=793) increased by a factor of 1966x and 40x, respectively. However, PPE was only a small portion (0.671% and 0.213%, respectively) of the total plastic litter reported. While PPE may contribute a small percentage to our plastic pollution problem, gloves and masks as litter have become a common sight and 37% of all cleanups reported PPE litter by the end of 2020. Monitoring the abundance of this type of environmental litter and the response of community volunteers will help us better understand the socio-ecological impacts of the current pandemic and inform policy for future pandemics.

08.06 Sustainability in Indigenous Food Systems

08.06.01 Tribal Food Sovereignty, Environmental Health, and Climate Change

N. Frank, NW Portland Area Indian Health Board; B. Jones, Columbia River Intertribal Fish Commission; V. Segrest, Consultant

The land, water, and air are our identity as native people and holds for us all the answers we need to be a healthy, vibrant, and thriving community. Without the elk, salmon, huckleberries, shellfish and cedar trees, we are nobody… This is our medicine; remembering who we are and the lands that we come from. In this session, we will discuss tribal food sovereignty and its intersections with environmental health and climate change. Many solutions are held within traditional knowledge and many tribal nations are leading efforts in healing the environment that sustain our food system. Food sovereignty can be defined as “The right of a people to healthy and culturally appropriate food that is produced through ecologically sound and sustainable methods, and their right to define their own food and agriculture systems.”

08.06.02 What Is Indigenous Agriculture?

N. Lincoln, University of Hawaii, Manoa / Tropical Plant and Soil Sciences

Using Hawai’i as a model socioecological system, this talk first examines the evolution of Native Hawaiian agriculture across the archipelago. The diverse ecosystems of Hawai’i offered various opportunities and constraints to agriculture, resulting in different systems of agriculture being developed. Alteration of the land further affected the decision of future descendants, creating intergenerational connections. Differences in requirements and yields of the various agricultural systems manifested in social, political, and cultural divergence between localities. Despite substantial differences in the forms of agriculture in ancient Hawai’i, care values and worldviews created certain commonalities that umbrella indigenous agriculture. This talk next explores perceptions about what are the essential elements of indigenous agriculture today. Emerging themes demonstrate that relationships - to oneself, to others, and to the environment - are of core importance. The talk concludes with an example of how we attempt to incorporate these themes in our own farm.

08.06.03 Case Study in Addressing Micro-Nutrient Deficiency: Zinc

E.J. Van Genderen, International Zinc Association / Director, Environment, Health & Sustainability; A.S. Green, International Zinc Association / Executive Director

The Copenhagen Consensus results from 2008 suggested that “Providing micronutrients for 80% of the 140 million children who lack essential vitamins in the form of vitamin A capsules and a course of zinc supplements would cost just $60 million per year…action holds yearly benefits of more than $1 billion”. Since then, the global zinc producing industry has sponsored >$10 million in activities to support health and agricultural aid towards international programs (e.g., UNICEF, USAID, etc.) and local Corporate Social Responsibility commitments to indigenous food security. While micro-nutrient fortification of staple foods and supplementation for children plays a critical role, zinc amendment in fertilizers has demonstrated improvements for crop yield, nutritional content, commercial growth, and societal outcomes. Activities to date have recognized indigenous needs through crop trials in China, Congo, India, Mexico, Peru, Turkey, among others, and linked cultural and societal benefits to positive economic and environmental conditions. With the world’s population set to exceed 9 billion people by 2050, food production must increase by 70% and addressing micro-nutrient deficiency is essential to improving food security.

08.07 Systems Approaches

08.07.01 Questions Remaining in the Quest to Quantify Ecosystem Services

A. Deines, Exponent / Ecological & Biological Sciences; R. Kashuba, EPA / Office of Research and Development; A. Morrison, Exponent; T. Newcomer-Johnson, USEPA / ORD

Ecosystem services provide metrics for quantifying the benefits of nature to humans as well as for measuring the consequences of human actions. For example, how much carbon is sequestered from a wetland mitigation project? How much carbon is released from a wildfire? Communicating the value of ecosystem components and functions in terms of services allows stakeholders to directly understand the intrinsic value of a natural system or compare the benefits and consequences of human activity. A major challenge in operationalizing this philosophy is developing methods of translating variable measures of environmental properties (data) into consistent quantitative estimates of the services they deliver. Ecosystem service estimates incorporate scientific uncertainty, such as sampling uncertainty, modeling uncertainty, and uncertainty associated with knowledge gaps, but also uncertainty in linking a measurable ecosystem property or function to the service it provides. Individual ecological production functions (EPFs) have been developed to answer specific questions in specific contexts, but ecosystem service metrics have not been defined consistently across EPFs in terms of space and time scales, model assumptions, and the portion of an ecosystem service addressed. For example, potential metrics in models quantifying inland wetland ecosystem services include properties like stream nitrogen load, denitrification rate, phosphorus retention, flood mitigation, and a variety of biological metrics (biodiversity, ecological functions supported, population sizes), as well as metrics of recreational benefits (birdwatching, hunting, and wetland aesthetics). Comparing available EPFs is a step towards harmonizing ecosystem service calculation and one difficultly is the different ways to scope and classify environmental properties, not only in terms of using different mathematical functions, inputs, and assumptions, but also in terms of what kind of ecosystem services it estimates. As the global economic community is looking to ecosystem services as a possible mechanism for accounting for the value of nature in business and social decisions, there is a need to understand the precision of ecosystem services estimates. Would two EPFs estimating flood mitigation services for the same area produce comparable estimates, even if they use different input data? This presentation will discuss the challenges we have identified regarding assessing such precision and open discussion for future research priorities.

08.07.02 The Environmental Burden of the Internet of Things: A Material Flow Analysis

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The Internet of Things (IoT) now comprises everything from smart lightbulbs to smart cities, from smart blood sugar detectors to smart military devices. New internet-connected, data-mining devices and sensors can be found at home, in healthcare centres, in industry – where they are part of the "fourth industrial revolution" – and beyond. Despite the increasing importance of the Internet of Things to industry and the global economy, no analysis has been done on the physical resources required to produce the various sensors, processors, and other components that go into each IoT device – let alone supporting infrastructure like data processing centres. Many of these components contain hard-to-find elements whose extraction is environmentally damaging, and whose deposits are often limited or are sources of geopolitical tension. Given the limited recyclability of the components, it is important to quantify how much of these elements are being consumed for IoT devices today to be able to predict future demands on resources. This information is intended to contribute to understanding the full life cycle impacts of IoT devices relative to their purported benefits – which for some devices includes improving energy efficiency through process optimization. We are conducting a material flow analysis of IoT devices in the home as a demonstration of the large quantities of elements and components that go into the IoT. The analysis identifies the sensors and components commonly found in archetypal IoT devices in the home, including smart lightbulbs, thermostats, and home assistant hubs. The quantities of major elements in these devices are estimated and then scaled to population-wide production and consumption estimates. We aim to draw attention to the environmental burden of IoT devices, recognizing that our analysis covers only a small portion of the "things" connected to the internet. Given the ever-expanding suite of IoT devices, and their increasing embeddedness in society, it is crucial that we have the information to manage the resources necessary for the system's devices before it becomes too late.

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