Presentation Order
Tuesday, March 15, 2016

10:00 A.M.	**Evaluation of the Status of Benthos and Plankton Communities at Wisconsin's Great Lakes Areas Of Concern on Lake Michigan** Barbara C. Scudder Eikenberry, Hayley A. Templar, Daniel J. Burns, Amanda H. Bell
10:20 A.M.	**Evaluating Impacts of Pulp and Paper Mill Process Changes on Bioactive Contaminant Loading to St. Louis Bay** Eric C Randolph, Gerald T Ankley, Jason P Berninger, Jenna E Cavallin, Evan P Eid, Megan Hughes, Michael D Kahl, Kathleen M Jensen, Joe Mayasich, Al Parrella, Anthony Schroeder, Daniel L Villeneuve, Kyle E Stevens
10:40 A.M.	**Surface Water Pharmaceutical Contamination: Investigating Adverse Biological Consequences in Fish Populations** Troy D Lehto, Heiko L Schoenfuss, Nicole M Bentz
11:00 A.M.	**Toxicity Prediction in Ecological Effects Analyses – Limitations, Uncertainty and Information Gaps** Tyler Linton, Keith Taulbee, Charles Delos, Wade Lehmann
11:20 A.M.	**Utilizing Hg Stable Isotope Ratios to More Fully Resolve Hg Sources and Processes in the Great Lakes** Ryan F Lepak, Runsheng Yin, David P Krabbenhoft, Thomas M Holsen, James P Hurley
11:40 A.M.	**Use of Air Dispersion Modeling In Predicting Risk from Beneficial Reuse of Steel Slags in Wisconsin** Adam B Streiffer, Robert L Thiboldeaux
1:00 P.M.	**Reproductive Effects of Pyriproxyfen on *Daphnia magna*** Benjamin Westerhoff, Heiko Schoenfuss
1:20 P.M.	**Impacts of Wastewater Contaminant Metformin on the Reproductive System of Fathead Minnows (*Pimephales promelas*)** Nicholas J Niemuth, Rebecca D Klaper
1:40 P.M.	**Chronic Effects Of Binary-Metal Mixtures Of Cadmium, Zinc, And Nickel On *Daphnia magna*** Edgar Perez, Tham Hoang
2:00 P.M	**On-Site Evaluation of Toxicity and Estrogenicity of Wastewater Effluents to the Endangered Rio Grande Silvery Minnow** Kevin J Buhl, Diana M Papoulias, Mandy L Annis, David A Alvarez, Travis W Schaeffer
2:20 P.M.	**Chronic Exposure of Fathead Minnows to a Complex Urban Mixture** Lina C Wang, Jessica L Ward, Heiko L Schoenfuss
2:40 P.M.	**Modulation of Estrogenic Effects via Temperature on Two Life Stages of *Pimephales promelas*** Megan K Cox, Jessica L Ward, Heiko L Schoenfuss
3:10 P.M.	**Persistent Lead Contamination in an Urban Marsh: The Legacy of Lead Shot** Tisha C King-Heiden, Colin Belby, Gretchen Gerrish
3:30 P.M.	**Does Social Hierarchy Modulate Responses Of Fish Exposed to Endocrine Disrupters?** Jelena Ivanova, Shiju Zhang, Heiko L Schoenfuss
3:50 P.M.	**Effect of Carbamazepine on the Phytotoxicity of Pharmaceutically Active Compounds** Madison E Czerwinski, Sara L Nason, Joel A Pedersen,
4:15 P.M	***Plenary Speaker:*** **Communicating Your Results After the Journal Paper: The Most Important Consumers of Your Hard Work Do Not Read Science Journals!** David Krabbenhoft, USGG

Presentation Order
Wednesday, March 16, 2016

8:20 A.M.	**Effects of Rearing Temperature And Polybrominated Diphenyl Ether (PBDE) on Growth, Development, and Metabolism of Leopard Frog (*Lithobates pipiens*) Tadpoles** Jeremiah M Yahn, William H Karasov
8:40 A.M.	**Detection of Avian Influenza A in Groundwater: Results from the 2015 HPAI Outbreak** Laura E Hubbard, Susan K Spencer, Mark A Borchardt, Joel P Stokdyk, Aaron D Firnstahl, Dana W Kolpin
9:00 A.M.	**Determining the Impacts of Toxics in the Great Lakes Using Genomic Biomarkers of Mussels involved in the Contaminant Monitoring of the NOAA Mussel Watch Program** Nicklaus Neureuther, Ed W Johnson, Kimani Kimbrough, Annie Jacob, Rebecca D Klaper
9:20 A.M.	**Acute Toxicity of Clothianidin in Zebra Finches: Development of a New Model of Neonicotinoid Toxicity In Passerines** Erik Hofmeister, Julia Lankton, Da Chen, Mark D. Jankowski
9:40 A.M.	**Contaminant Exposure in Tree Swallows Nesting Along the St. Louis River, Duluth, MN and Superior, WI** Christine M Custer, Thomas W Cyster, Paul M Dummer
10:00 A.M.	**Ingestion of Microplastic Associated with Green Algae by *Daphnia magna*: Evidence of No Effect** Patrick M. Canniff, Tham C Hoang
10:35 A.M.	**The Roles of Physicochemical Properties and Root Physiology In Predicting Plant Uptake of Pharmaceuticals and Personal Care Product Ingredients** Elizabeth L Miller, Sara L Nason, K G Karthikeyan, Joel A Pedersen
10:55 A.M.	**Demonstration of Scalable Analytical Methods for the Screening of Algae Bloom Contaminated Surface Waters by UHPLC-TOFMS Equipped With A Novel And Automated Analyte Search Algorithm** Frank Kero, Nicole Lenca, Judy Westrick
11:15 A.M.	**Metabolomics: Dose Response in Cellular Assays** Joel G Putnam, Justine E Nelson, Eric Leis, Richard A Erickson, Terrance D Hubert, Jon J Amberg
11:35 A.M.	**Bringing the Fathead Minnow into the Genomic Era** Travis W Saari, Daniel L Villeneuve, Gerald T Ankley, Frank R Burns, Amarin L Cogburn, Stephane D Deschamps, Raymond E Jackson, Robert A Hoke, Anthony L Schroeder
11:55 A.M.	**Polybrominated Diphenyl Ethers (PBDES) in Fish From Wisconsin's Great Lakes and Inland Waters** Meghan CW Williams, Candy Schrank

POSTER ABSTRACTS

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2	**A Refined Multi-Site Model to Estimate the Toxicity of PAH-Contaminated Sediments at MGP Sites**	Michael W Kierski, Ann Michelle Morrison, Susan B Kane Driscoll, Charles A Menzie
3	**A Survey: Demographics and the Rebound Effect in Vehicle Fuel Efficiency**	James M Mahoney, Andrea L Hicks
4	**Adsorption of Lamotrigine to Montmorillonite Clay**	Brian Ferrer, Sara Nason, Joel Pedersen
5	**Assessing the Conventional Calculated Method (CCM) of the Globally Harmonized System (GHS) for Acute Aquatic Toxicity Classifications of Chemical Mixtures Using 48-Hour LC50 *Daphnia magna* Test Results**	Kim D'Aloia, Magdalena Osorio, Owen Kinsky, Nathan Pechacek
6	**Biological Effects of Septic Pollution on Larval Fathead Minnows, *Pimephales promelas***	Les Warren, Heiko Schoenfuss
7	**Assessing the Influence of Part Per Billion Variation of Natural Organic Carbon Levels on Cationic Polymer Acute Toxicity to *D. magna***	Jared S Bozich, Edward R Salinas, Lars Peters, Reudiger Lukas, Rebecca D Klaper
8	**Association of the Anticonvulsant Drug Lamotrigine With Dissolved Humic Acid**	Bei Liu, Joel A Pedersen
9	**Bacterial Transformation of Engineered Nanomaterials Used in Electric Vehicle Energy Storage Devices**	Debra R Garvey
10	**The Fate of Fragrance Chemicals from Wastewater Treatment Plants**	Helaina Rosenmayer, Macy Anderson, Sarah A Rubinfeld
11	**Changes in Tyrosine Hydroxylase Expression In Zebrafish Embryos Resulting from Exposure to Bisphenol A (BPA)**	Brenda L McKee, Sarah Gempeler, Kelsey Smith, Peter Kuhn
12	***In Vitro* Assessment of Androgenic Activity of Water Reclamation Plant Effluents in Greater Chicago**	Abigail Lukowicz, Jackie Heitzman, Tom Minarik, Dalma Martinović-Weigelt
13	**Effects of the Azole Fungicide Imazalil on the Fathead Minnow (*Pimephales promelas*) Steroidogenesis Pathway**	David J Feifarek, Rebecca Y Milsk, Kathleen M Jensen, Brett R Blackwell, Eric C Randolph, Jenna E Cavallin, Travis W Saari, Michael D Kahl, Gerald T Ankley, Wan-Yun Cheng, Daniel L Villeneuve
14	**Empirical Models for Quantifying the Role of Chemical Pollutants of Emerging Concern in Fish Biodiversity Loss in US**	Daniel Bampoh, Shweta Singh
15	**Evaluating Chemical Tracers In Suburban Groundwater As Indicators of Nitrate-Nitrogen Sources**	Amy L Nitka, William M DeVita, Paul M McGinley
16	**Evaluation of Traditional Soil Testing Methods to Estimate Lead Hazard In Soil**	Shannon A Plunkett, Douglas J Solda
17	**Evaluation of Whole-mount *In Situ* Hybridization as a Tool for Pathway-Based Toxicological Research in Early-Life Stage Fathead Minnows**	Jenna E Cavallin, Anthony L Schroeder, Brett R Blackwell, Kerri Carlson, Kathleen M Jensen, Michael D Kahl, Eric C Randolph, Daniel L Villeneuve, Gerald T Ankley
18	**Evidence of Lampricide Photodegradation During Field Applications to Tributaries of the Great Lakes**	Megan B McConville, Adam S Ward, Christina K Remucal
19	**Exposure to two 2,4-D Herbicide Formulations Decreased Larval Survivorship in Fathead Minnows (*Pimephales promelas*)**	Gavin K Dehnert, Mariella B Freitas, Zachary A DeQuattro, Terence P Barry, William H Karasov
20	**Examining the Demographic Effects of Coal Fly Ash Exposure Routes in *Daphnia magna* and *Daphnia pulex***	Madison Hull, Paul C Pickhardt
21	**Impacts of Early Exposure to Triclosan on Growth, Maturation, and Reproduction in Zebrafish**	Cole W Fuchs, Tisha King-Heiden

22 **The Effects of Chlorinated Solvents on Tyrosine Hydroxylase 1 Expression In Zebrafish Embryos Using Quantitative Polymerase Chain Reaction (qPCR)**
Brenda L McKee, Emilie Anderson, Emily Buchner, Caroline Kucha, Sarah Datza, Sarah Rendon, Peter Kuhn

23 **Interaction of Nanoparticles with Gram-Positive Bacterial Surfaces**
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24 **Tree Swallows as Indicators of Sediment Contamination**
Thomas W Custer, Christine M Custer

25 **Population Effects of Coal Fly Ash-Exposed *Chlamydomonas reinhardtii* Fed to *Daphnia magna* at Low and High Food Concentrations**
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26 **Defining Great Lakes Tributary Representative Mixtures For Chemical Exposure Experiments**
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27 **Chronic Effects of Lead to Topsmelt Fish (*Atherinops affinis*); SSD Development, Influence of Salinity, and Influence of Organism Age Enhancement of Microplastics on Endosulfan Sulfate Uptake in Earthworm (*Lumbricus terrestris*)**
Erik Reynolds, Tham C Hoang

28 **Enhancement of Microplastics on Endosulfan Sulfate Uptake in Earthworm (*Lumbricus terrestris)***
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29 **Characteristics of Wisconsin Male Anglers over 50 Related to Fish Consumption and Advisories**
Candy Schrank, Meghan Williams, Krista Y Christensen, Henry A Anderson

30 **Method Refinements for the Midge Life-cycle, *Chironomus dilutus* Test**
Teresa J Norberg-King, Terry L Highland, J Russell Hockett, David R Mount

Evaluation of the Status of Benthos and Plankton Communities at Wisconsin's Great Lakes Areas of Concern on Lake Michigan

Barbara C Scudder Eikenberry[1], Hayley A Templar[1], Daniel J Burns[1], Amanda H Bell[1]

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The United States and Canada designated 43 Areas of Concern (AOCs) in the Great Lakes Water Quality Agreement (GLWQA) of 1972 and amendments, and four of Wisconsin's five AOCs lie adjacent to Lake Michigan. AOCs are severely degraded areas that fail to meet objectives of the GLWQA because of the presence of at least 1 of 14 Beneficial Use Impairments (BUIs), including BUIs for degradation of benthos (benthic invertebrates) and degradation of plankton (zooplankton and phytoplankton) communities. These two BUIs may be removed if a community at an AOC has improved enough that it is not statistically different from a community at a selected reference area. To inform management decisions regarding removal of these two BUIs, the U.S. Geological Survey collected samples to quantify benthos and plankton communities at the four AOCs and six less-impacted comparison sites ("non-AOCs") three times per year in 2012 and again in 2014. The four AOCs included in this study are the Lower Menominee River, Lower Green Bay—Fox River, the Sheboygan River, and the Milwaukee Estuary (including sites in the Milwaukee Harbor, Milwaukee River, and Menomonee River), and each AOC was paired with two non-AOCs of similar size and land use. Relative abundance and selected metrics (richness, diversity, and an Index of Biotic Integrity [IBI] for benthos only) were statistically compared between each AOC and all nonAOCs as well as between each AOC and its two paired nonAOCs. Results indicate that the benthos IBI at the Lower Menominee River was significantly lower than at its paired nonAOCs in 2012 and 2014 and was therefore deemed degraded. Similarly, benthos richness and the benthos IBI at the Milwaukee Estuary was deemed degraded in 2012 but not 2014; zooplankton richness was deemed degraded at the Fox River and Milwaukee Estuary in 2012 only and at the Sheboygan River in 2014 only. Although phytoplankton richness and diversity at the AOCs were not deemed degraded in 2012, phytoplankton richness and diversity at the Fox River was deemed degraded in 2014. State governments, citizen groups, and the USEPA will use results of these studies to monitor future improvements as well as input to evaluate whether recent remediation efforts have been effective and the BUIs can be removed.

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Evaluating Impacts of Pulp and Paper Mill Process Changes on Bioactive Contaminant Loading to St. Louis Bay

Eric C Randolph[1], Gerald T Ankley[1], Jason P Berninger[2], Jenna E Cavallin[3], Evan P Eid[1], Megan Hughes[1], Michael D Kahl[1], Kathleen M Jensen[2], Joe Mayasich[4], Al Parrella[4], Anthony Schroeder[5], Daniel L Villeneuve[1], Kyle E Stevens[1]

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As a convergence point for human waste streams, wastewater treatment plants are recognized as point sources through which contaminants originating from domestic, industrial, and commercial activities enter surface waters. Effluent from the Western Lake Superior Sanitary District (WLSSD), which discharges into the St. Louis River (MN and WI, USA), has previously been shown to exhibit both estrogenic and aryl hydrocarbon receptor-mediated bioactivities. A long-standing question has been the relative contribution of domestic (accounting around 55% of inflow) versus industrial sources like the Sappi Pulp and Paper Mill (SPMM; accounting around 45% of inflow) to these bioactivities. During the summer of 2013 SPPM underwent a series of process changes which could potentially influence the contaminant loading into WLSSD. Consequently, a series of effects based monitoring studies on final treated effluent and receiving water were conducted pre-, during, and post SPPM process changes to determine whether these changes in processes markedly affected the bioactivity associated with the effluent. Fathead minnows were exposed to a constant flow of effluent of varying concentrations (100%, 20%, 5%) on site at WLSSD or caged within the St. Louis River receiving waters. Estrogenic activity, as determined using in vitro bioassays, was not markedly altered by the SPPM process changes. Additionally, consistent with previous years, no major impacts on steroid biosynthesis were evident. Hepatic expression of vitellogenin (vtg) mRNA was consistently elevated in males exposed to 100% effluent. This elevation of vtg mRNA was not seen in males exposed to 20% or 5% effluent. The relative abundance of Cytochrome P4501A1 mRNA showed induction in a dose dependent manner between treatment groups for all time points. Analysis of water samples from the different exposure periods yielded the detection of 70 organic contaminants within the final treated effluent. Based on these results, the hypothesis that a change in the pulping process at SPMM would affect contaminant loading from WLSSD into the St. Louis Bay was not supported.

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Surface Water Pharmaceutical Contamination: Investigating Adverse Biological Consequences in Fish Populations

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The field of Aquatic Toxicology has produced diverse studies on Contaminants of Emerging Concern (CECs). Investigations have brought to light a plethora of knowledge on the subject; of particular interest are those involving pathways by which CECs arrive in waterways (source), the concentrations which they exist therein (fate), and to what extent they affect aquatic animals (effect). Particularly concerning CECs are pharmaceuticals that find their ways into aquatic environments as these compounds are inherently biologically active and stable in aqueous solutions. Interest in potential adverse effects on exposed animals has begun to yield compelling evidence supporting behavioral and developmental disturbances as physiological and anatomical pathology receive increased attention. In an attempt to identify anomalies as a consequence of pharmaceutical contamination, several exposure experiments were accomplished. Larval fathead minnows (Pimephales promelas) and the common bluegill panfish, Lepomis macrochirus, were utilized in 20-day static exchange and 30-day flow-through designs, respectively. The pharmaceuticals being investigated were those representing multiple modes-of-action and include diclofenac, methocarbamol, sulfamethoxazole, and temazepam. The pharmaceuticals were administered at concentrations measured downstream of wastewater treatment plant effluent discharges. Identification of variation between treatment groups involved analysis of multiple data sets including morphometric measurements and behavioral characteristics, as well as tissue and fluid-based physiological assays. No overt mortality was observed and analysis of behavioral and morphological assays is ongoing.

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Toxicity Prediction in Ecological Effects Analyses – Limitations, Uncertainty and Information Gaps

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The acquisition of toxicity test data of sufficient quality from open literature to fulfill taxonomic diversity requirements can be a limiting factor in ecological effects analysis. Existing toxicity prediction models (WebICE) or conventions (ACRs) can be used to estimate acute and chronic effects endpoints at the species, genera, or family level for those taxa without test data, but the effectiveness of the predictive tool or convention is contingent on the quantity and quality of the empirical data used to generate the estimates, and thus could benefit from sort of data prioritization process that considers degree of data certainty. We will introduce a conceptual framework for considering risk to species with a paucity of biological effects data based on empirical and predicted acute and chronic toxicity values, and discuss some limitations, uncertainties and key information gaps in the context of ecological effects assessment. *Note: This presentation may be subject to approval from EPA*.

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Utilizing Hg Stable Isotope Ratios to More Fully Resolve Hg Sources and Processes in the Great Lakes

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Multi-collector inductively coupled plasma mass spectrometry of stable mercury (Hg) isotopes can be used to track sources and understand key cycling pathways. Similar to sulfur isotopes, Hg may undergo a large range of mass-dependent (MDF) and mass-independent fractionation (MIF) in ambient conditions. Some of the important reactions affecting MDF include redox transformations, biological cycling, and volatilization. In addition, photochemical demethylation of methylmercury and photo-reduction of Hg(II) results in odd MIF. While MIF is reportedly rare in heavy metals in the environment, (Hg and U have been reported) this characteristic provides the opportunity for multidimensional tracers to further fingerprint specific chemical pathways. These combined measurements and insights have allowed for focused Hg source tracking studies and helped elucidate Hg processing pathways, providing a new tool for studying Hg cycling in the environment. A recent study using Hg stable isotopes revealed the three primary contributors of Hg to Great Lakes sediment: atmospheric, watershed derived, and industrial. An isotopic mixing model, based on MDF and odd MIF (δ202Hg and Δ199Hg), provided evidence of the sources of Hg to surface sediments of each lake. In addition, anomalous even MIF (Δ200Hg), detectable in sediment, was used to independently confirm the primary model presented. Previous research suggests both that even MIF is conserved during biogeochemical processing and odd MIF is conserved during metabolic processing. It is reasonable to suspect that even MIF is similarly conserved through metabolism. Preliminary data suggest that even MIF may be used as an indicator of atmospheric sources of Hg and if isotopically conserved, an indicator of atmospheric sources during bioaccumulation. While we are expanding our isotopic analyses to include lake trout (Salvelinus namaycush) samples collected over time in the Great Lakes to assess the usefulness as a tracer, it is important to confirm even MIF signal preservation through various trophic levels in the Great Lakes. Potentially, isotopic Hg signatures can be used for natural resource management and restoration decisions, but a better understanding of relevant transport and transformation processes and effects on Hg isotope distribution for lacustrine systems is needed.

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Use of Air Dispersion Modeling In Predicting Risk from Beneficial Reuse of Steel Slags in Wisconsin

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The Wisconsin Department of Health Services (DHS) assessed human health risks from the beneficial use of electric arc furnace (EAF) slag, ladle slag and the mixture of EAF and ladle (mixed) slags from a Wisconsin steel-production facility for unconfined uses in Southeast Wisconsin. DHS collaborated with the Department of Natural Resources' (DNR) air modelers to predict air concentrations and subsequent inhalation exposure that might result from the application of the slags as road bedding and parking lot construction material. These modeled results were then applied to standard human health risk assessment methods to predict inhalation health risk. Additionally, we estimated the health risks of child incidental ingestion exposure from the use of slag in residential driveway slag applications. Exposure risks were estimated based on the metallic profile of the slags using facility-specific data provided by DNR. Several scenarios were evaluated using assumptions reflecting seasonal exposure conditions, as well as consideration of the bioavailability of metals via ingestion. From the risk estimates, DHS concluded that unhealthy exposures to children via direct contact may reasonably result from use of slag on residential driveways and access roads. However, a worst-case exposure analysis of modeled ambient fine particulate (PM10) concentrations produced by traffic on roads, shoulders and parking lots constructed of unconfined slag failed to indicate health hazards from constituents of the slag in the PM10 fraction. The conclusions support the recommendations that the slags be restricted in residential settings, but that no restrictions are required for road shoulder and parking lot construction applications.

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Reproductive Effects of Pyriproxyfen on *Daphnia magna*

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Contaminants of emerging concern (CEC's), including pyriproxyfen, are widely distributed in aquatic ecosystems. Unlike many CEC's, pyriproxyfen is intentionally introduced to aquatic ecosystems to control mosquitos. Exposure to this compound has been shown to affect reproduction by increasing the occurrence of males in parthenogenetically reproducing D. magna. In this study, we conducted exposures with multiple concentrations of pyriproxyfen to assess the concentration of effect (EC10) of this pesticide. D. magna were exposed for 16 days post-hatch to pyriproxyfen. Neonates produced by exposed organisms were observed under magnification to determine sex ratios. Exposure concentrations were 29, 59, 119, 179, 209, and 239 ng/L pyriproxyfen. Our results suggest that *D. magna* have an EC10 at 119 ng/L. This was a pilot study for future work that will look for changes in RNA expression when *D. magna* are exposed to CEC's like pyriproxyfen.

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Impacts of Wastewater Contaminant Metformin on the Reproductive System of Fathead Minnows (*Pimephales promelas*)

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The discovery of intersex fish in freshwater systems around the world suggests the widespread presence of endocrine disrupting compounds in the aquatic ecosystem, likely from human waste. Synthetic estrogens from birth-control medications found in wastewater have been pointed to as one likely cause of observed endocrine disruption in the environment. However, many other pharmaceuticals are being discovered in wastewater and surface waters at small but significant concentrations, and their impacts on aquatic organisms at environmental concentrations remain largely unknown. The anti-diabetic drug metformin is prescribed worldwide as a treatment for type 2 diabetes. This biguanidine is excreted in human waste in its active form and, although it is removed to a high degree by wastewater treatment, recent sampling of effluent and surface waters around the world have revealed the ubiquitous presence of this compound, believed to be the pharmaceutical most deposited into the aquatic environment by mass. Importantly, metformin is also prescribed to treat hormonal imbalances in patients with polycystic ovarian syndrome, an endocrine disorder, suggesting its potential as an endocrine disrupting compound in the environment. We exposed fathead minnows (Pimephales promelas) from fry stage to adulthood to a concentration of metformin found in wastewater effluent and examined reproductive, histopathological, and gene-expression endpoints to determine any potential endocrine-disrupting effects of exposure to this widely found contaminant.

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Chronic Effects of Binary-Metal Mixtures of Cadmium, Zinc, And Nickel On Daphnia magna

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Metals are present in the natural environment as a mixture of multiple elements. Concern about metal mixture effects is growing. This study characterized binary-metal mixture effects of cadmium (Cd) and zinc (Zn) and Cd and nickel (Ni) on *Daphnia magna*. The titration design was selected to characterize the 21-d chronic effects of the binary metal mixtures on survival, growth, reproduction, and metal accumulation in D. magna. Using this design, increasing concentrations of Zn (10, 20, 40, 80, 120, 160 and 200 µg/L) and Ni (20, 40, 80, 100, 120, 140, and 160 µg/L) were titrated against a constant concentration of 1.5 µg/L Cd. The results demonstrate that Cd was highly toxic to *D. magna*. In a mixture with Cd and Zn, concentrations of 10 and 20 µg/L Zn were consistently insufficient to induce total protective effects (e.g., 100% survival) from Cd toxicity to *D. magna*. Conversely, mixtures containing 40, 80, and 120 µg/L Zn provided strong protective effects to *D. magna* at all endpoints. On the other hand, Ni concentrations of 20, 40, and 80 µg/L partially protected *D. magna* from Cd toxicity but only at the survival and growth endpoints. Additionally, no nickel concentration was observed to provide total protective effects to *D. magna*. Embryos analyzed for morphological differences demonstrate severe developmental defects in both binary- metal mixtures. The results of the present study are useful for development of environmental quality guidelines for metal mixtures.

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On-Site Evaluation of Toxicity and Estrogenicity of Wastewater Effluents to the Endangered Rio Grande Silvery Minnow

Kevin J Buhl[1], Diana M Papoulias[2], Mandy L Annis[2], David A Alvarez[2], Travis W Schaeffer[1]

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Two 21-day on-site toxicity studies were conducted with juvenile Rio Grande silvery minnows. One study exposed silvery minnows in cages deployed in the effluent channel of three municipal wastewater treatment plants (WWTP) in the Middle Rio Grande Basin, NM, and in Middle Rio Grande. The second study exposed fish in the laboratory to the same effluents and river water along with a positive control (5 ng/L ethynylestradiol, EE2) and solvent control (ethyl alcohol). There were four replicates per site/treatment with 10 fish in each replicate. At the end of exposures, fish were measured for total length and weight and frozen for analysis of whole-body vitellogenin (Vtg). Exposure conditions were characterized by in situ monitoring of water quality using sondes, ex situ analysis of general water quality parameters, and polar organic chemical integrative samplers (POCIS). The POCIS were configured to sample a suite of organic wastewater-related contaminants. Survival of caged-exposed fish in the river and two effluent sites was not significantly different and ranged from 82.5% to 97.5%. An incident occurred at the other WWTP that resulted in 30-100% mortality on day 11. Caged fish in the river and one effluent site lost weight (6%), whereas those in the other two effluent sites gained weight (44-63%) and were significantly heavier than those in the river. In the laboratory exposures, there were no significant differences in survival or growth of fish among the six treatments. Fish exposed to 5 ng/L of EE2 for 21 days had significantly greater whole-body Vtg concentrations than those in any of the other treatments. Whole-body Vtg concentrations in fish exposed to the effluents in cages or in the laboratory were not significantly different from the river water or solvent control treatments. Results indicate that compounds present in these effluents did not elicit significant Vtg induction in juvenile silvery minnows.

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Chronic Exposure of Fathead Minnows to a Complex Urban Mixture

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Aquatic organisms exposed to contaminants of emerging concern (CECs) may have reduced reproductive fitness via disruption of reproductive and non-reproductive behavioral and physiological pathways. In the laboratory, larval and mature fathead minnows (Pimephales promelas) were exposed to a high (10x higher than environmental), medium (environmental), and low (10x lower than environmental) concentrations of an urban CEC mixture derived from actual Great Lakes Area inputs (BPA, DEET, TBEP, Methyl-1H-benzotriazole, HHCB, Estrone, Sulfamethoxazole, Desvenlafaxine, Fexofenadine, and Metformin). Behavioral assays, assessing exposure-induced changes in aggression, courtship, boldness and feeding rate were conducted for reproductively mature minnows. In addition, fecundity and early survival endpoints were also recorded. C-start predator escape behaviors, rheotaxis and foraging ability were assessed for larval minnows exposed for 21-days post-hatch. Data analyses are ongoing, but our preliminary results indicate that adult female minnows exposed to environmental mixture concentrations show a significantly lower feeding rate, compared to control fish. No significant difference in liver or gonad weight was observed among fish from the different treatments. The alteration of apical endpoints sheds additional light on how CECs in aquatic waterways can adversely impact fish populations, and highlight the importance of considering multiple life stages when assessing contaminant interactions.

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Modulation of Estrogenic Effects via Temperature on Two Life Stages of *Pimephales promelas*

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Contaminants of emerging concern, including estrogens, are widespread in aquatic environments. One way that these contaminants are introduced into aquatic ecosystems is via effluent discharged from wastewater treatment plants. Exposure to wastewater estrogens has been shown to adversely affect the survival and behavior of fish, which could impair the sustainability of exposed populations. However, assessing the effects of estrogen exposure on individuals is complicated by the fact that rates of chemical uptake and environmental degradation, are temperature dependent, and thus likely to be seasonally variable. In this study, a factorial experiment was conducted to assess the influences of temperature and estrogen concentration on two life stages (i.e., larval and adult) of fathead minnow (P. promelas). Minnow eggs and larvae were exposed to three concentrations of estrone (25, 125, and 625 ng/L) or to an ethanol carrier control (0 ng/L), at four water temperatures (15, 18, 21, and 24 °C) for 21 days, using a 50% daily static renewal protocol. Adult minnows were exposed to three concentrations of estrone (25, 125, and 625 ng/L) or to an ethanol carrier control (0 ng/L), at four water temperatures (15, 18, 21, and 24 °C) for 30 days, using a flow-through exposure system. A suite of behavioral and physiological endpoints were collected for larval and adult stages, including predator evasion, nest aggression, survival, and fecundity. The results indicate that temperature has a significant but unpredictable modulating effect on the physiological and behavioral effects of exposure.

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Persistent Lead Contamination in an Urban Marsh: The Legacy of Lead Shot

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The La Crosse River Marsh (LRM) is a unique urban riparian marsh recognized for its high biodiversity in an urban setting. The La Crosse Gun Club operated a large trap shooting range at the LRM between 1932-1963, resulting in significant quantities of lead (Pb) shot being discharged into the marsh. Pb-contamination remains high as we have found Pb shot densities of >43,000 pellets/m2, and surface sediments contain as high as 23,000 ppb Pb in some areas of the marsh. Using a combination of field and laboratory studies, we used a multi-disciplinary approach to survey and assess the impacts these contaminated sediments have on the marsh ecosystem. Water in the marsh contained concentrations within the range of concern for chronic toxicity. Pb from contaminated sediments is bioavailable, and Pb levels within duckweed, invertebrates, and some fish species correlate with the Pb found within sediments. Our laboratory toxicity assays suggest that sediments are minimally toxic to developing fish following acute exposure. Since the lead is not mobilizing system wide, we suggest any remediation efforts be focused in specific areas of high concentrations of the LRM, and that continued monitoring of the marsh is warranted.

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Does Social Hierarchy Modulate Responses of Fish Exposed To Endocrine Disrupters?

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Numerous studies have investigated the effects of laboratory fish exposures to endocrine disrupters, often using male fathead minnows (Pimephales promelas) as test organisms. Among the biological endpoints commonly evaluated in these studies is the egg-yolk precursor protein vitellogenin (VTG), which is expressed in male fishes exposed to estrogenic compounds. However, male fish in single-sex exposure groups establish social hierarchies, which are under androgenic control. As a result, more dominant fish develop more pronounced secondary sex characteristics (SSCs) due to higher circulating androgen concentrations with opposite effects in subordinate males. We conducted a meta-analysis of male fathead minnow exposure studies to determine whether fish would fall into distinct subpopulations based on their social status, and whether status influences the response to estrogens by modulating VTG synthesis. We pooled ten studies, all utilizing fish from the same breeding colony and conducted in the same exposure laboratory and were exposed to the same chemical compounds. Initially, only fish from control treatments were used to build the statistical model to eliminate the treatment effect. On average, the sample size of each treatment consisted of ten fish, and each experiment utilized in this study contained duplicates of each treatment. One-way ANOVA was run on the environmental conditions, such as temperature, pH and dissolved oxygen to assure similarity in exposure conditions across all pooled experiments. Multicolinearity issues were resolved using principal component analysis (PCA), which also helped to reduce the number of variables used in the cluster analysis. K-Means cluster analysis separated the fish population into three distinct groups. MANOVA was run on the obtained clusters and identified the significant differences between them. The results indicated that fish with the lowest SSCs also synthesized the lowest amount of VTG, fish with the highest SSCs had the second lowest levels of VTG, whereas fish with intermediate SSCs had the highest plasma VTG concentrations. These finding suggest that social status may modulate effect on fishes exposed to endocrine disrupters. They also suggest that fish of intermediate social status are the most susceptible to effects of endocrine disrupters.

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Effect of Carbamazepine on the Phytotoxicity of Pharmaceutically Active Compounds

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Crops irrigated with reclaimed wastewater (RWW) or grown in soil amended with biosolids can be exposed to pharmaceutically active compounds (PhACs) due to their inefficient removal in conventional wastewater treatment processes. PhACs typically occur as mixtures in RWW, but little information exists on how drug interactions affect plant uptake and metabolism of these compounds. Accurately determining crop plant uptake of PhACs and subsequent human exposure to PhACs and their metabolites requires a thorough understanding of in planta metabolism. In humans, the anti-convulsant drug carbamazepine (which is commonly found in RWW) induces several cytochrome P450 enzymes (CYP450s) that increase drug metabolism, reducing the effectiveness of other pharmaceuticals. Plants also have CYP450s that are responsible for contaminant metabolism. Although sequence homology between plant and mammalian CYP450s is limited, carbamazepine may affect the ability of plants to metabolize other PhACs. Preliminary results from root length assays show that the presence of carbamazepine may reduce the phytotoxicity of other pharmaceuticals to the model plant Arabidopsis thaliana.

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Effects of Rearing Temperature And Polybrominated Diphenyl Ether (PBDE) on Growth, Development, And Metabolism of Leopard Frog (Lithobates pipiens) Tadpoles

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Persistent organic pollutants (POP), such as PBDEs and PCBs, pose a serious threat to amphibian populations within the Great Lakes. Various studies have shown that chronic dietary exposure to POPs can affect growth and development of tadpoles. However, further investigation is needed to address the mechanism behind these effects as well as how these patterns behave with changing temperatures. In a 2X2 design, we raised tadpoles from embryos at either 22 or 27 ^{o}C on a control diet, and then at day 17 post-hatch (approx. Gosner stage (GS) 27) we began feeding them food either with or without a commercial mix of PBDE congeners (DE-71; 100 ng/g wet food). Tadpoles developed (increased in GS) and grew (increased in mass) slower at cooler rearing temperature and in the presence of PBDE. Therefore, we tested whether slower growth in tadpoles fed PBDE might be due to an effect of PBDE on resting metabolic rate (RMR), measured as O_2 consumption. Oxygen consumption was measured at the temperature the tadpoles were reared as well as the corresponding warmer or cooler temperature, to test for the effect of acclimation. Between GS 30-40 (masses approx. 1 to 6.6 g, n=66 tadpoles), results from an ANCOVA revealed a significant effect of ln(mass) ($P < 0.0001$) and rearing temperature ($P < 0.0001$), on ln(RMR), with no significant effect of PBDE exposure on ln(RMR) ($P>0.7$). Apparent Q10 values (rate change for 10o change in temperature) were approximately two for the effect of rearing temperature and approximately 1.5 for the effect of testing temperature in each group. We conclude that slower growth and development in tadpoles exposed to PBDE is not due to proportionally greater allocation of ingested energy to respiration, but more likely to lower feeding or digestion rate. The data on respiration and production at different temperatures can be used to improve predictions of effects of warming climate on tadpole energetics and ecotoxicology. *Funding provided by Sea Grant College Program, NOAA (Grant no. NA10OAR4170070, Project R/HCE-14).*

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Detection of Avian Influenza A in Groundwater: Results from the 2015 HPAI Outbreak

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Highly pathogenic avian influenza (HPAI) infected poultry in the Midwestern US in spring of 2015. By the end of June 2015, this outbreak resulted in 48 million dead birds in 15 states, with roughly 67% of the impacted birds (32 million) in Iowa. Wild birds are known to play a role in virus transmission, but the role of environmental reservoirs in the persistence and transmission of the virus is not well understood. HPAI has been documented in surface water proximal to infected poultry operations; however, we are unaware of research that has investigated HPAI in groundwater. In response to the 2015 outbreak, a pilot study was conducted to determine the viability of groundwater as a source for HPAI transmission. Samples were collected from groundwater (20 wells) and surface water structures (6 lagoons and 1 pond) in 15 outbreak-impacted poultry facilities in Iowa and Wisconsin. Facilities covered a range in geography, well depths (24 to 158 meters), facility type (pullet, table egg, commercial meat, backyard), facility size (4 thousand to almost 4 million birds), and time elapsed since onset of outbreak (8 to 79 days). Four of the 27 samples (three wells and one lagoon) tested positive for the avian influenza A matrix gene using qPCR, which indicates the presence of the avian influenza virus but does not confirm presence of the outbreak subtype (i.e., H5). H5 subtyping using the National Veterinary Services Laboratory standard protocol primers were negative. A semi-nested qPCR assay specific to the H5 HPAI outbreak strain was subsequently developed using the consensus H5 genetic sequence from the 2015 outbreak isolates. Using this test, one lagoon and one well were positive, suggesting the presence of HPAI. In addition, seven wells (35%) were positive for the poultry-specific parvovirus, confirming poultry-related virus transport pathways between poultry fecal waste and groundwater. Our research suggests that H5 was likely transported to groundwater underlying an HPAI infected poultry operation.

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Determining the Impacts of Toxics in the Great Lakes Using Genomic Biomarkers of Mussels involved in the Contaminant Monitoring of the NOAA Mussel Watch Program

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The health of the benthic community is critical not only for the ecosystem health of the Great Lakes, but impacts on benthic organisms are also part of the Beneficial Use Impairment (BUIs) for Areas of Concern. NOAA's NCCOS Mussel Watch Program monitors chemical pollution in the near shore zones of the Great Lakes and under the Great Lakes Restoration Initiative has included measures in Areas of Concern (AOC) around the Great Lakes. Our research builds on the chemical presence information by adding information on the health of mussels within these AOCs versus other long-term reference sites. This includes a multi-agency project in the Niagara River AOC. Contaminants in this river system include PCBs, PAHs, pesticides and chemicals of emerging concern. In this project we examined genomic biomarkers related to stress, reproduction, and general physiology in dreissenid mussels in sites that have been remediated to compare to reference and heavily contaminated sites to determine if remediation efforts impact the health of mussel species. Data indicate that a combination of biomarkers rather than single biomarkers provide an indication of contamination and stress and relate to remediation efforts.

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Acute Toxicity of Clothianidin In Zebra Finches: Development of a New Model of Neonicotinoid Toxicity In Passerines

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Neonicotinoid insecticides are nicotinic acetycholine receptor agonists and are highly effective and commonly used in agriculture as seed coatings. However, while these compounds were developed to be insect-specific, they have been associated in birds with acute death from consumption of coated seeds and with reproductive and immune system abnormalities in laboratory exposed birds. Our overall goal is to establish a model for acute and chronic neonicotinoid toxicity in a passerine in order to study reproduction and immunity in potentially exposed wild birds. As a first step, we conducted an oral acute toxicity trial in adult zebra finches using clothianidin (CTD) at 5 levels from 2 – 2000 mg/kg body weight with 8 birds per treatment level. Birds were euthanized 14 days post-exposure or as necessitated by clinical signs. In birds treated with ≥ 200 mg/kg, clinical signs of toxicity began as soon as 15 min after treatment and included respiratory distress and an inability to fly or to retain an erect posture that increased in severity in a dose-dependent manner. Four birds treated with 2000 mg/kg CTD were euthanized 6 hr after treatment due to the severity of their clinical signs. By 24 hr after treatment, most of the remaining birds had returned to flight, perching, and normal behavior. We estimated the LD50 for CTD was <2000mg/kg and the lowest observed effect level (LOEL) was 200 mg/kg. Histopathology revealed an increased vacuolization of hepatocytes in acutely affected birds and birds sacrificed at 14 days that were treated with 1000 – 2000 mg/kg CTD. Using a standard spectrophotometric assay, the mean acetylcholinesterase (AChE) activity in acutely affected birds and in birds sacrificed at 14 days that were treated with 200 – 2000 mg/kg CTD was significantly higher than the mean activity for control birds, suggesting increased AChE activity may be a biomarker for neonicotinoid toxicity, although studies with other neonicotinoids and bird species are needed. This study sets the range of CTD that might be used in a chronic toxicity trial in zebra finches.

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Contaminant Exposure In Tree Swallows Nesting Along the St. Louis River, Duluth, MN and Superior, WI

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The Great Lakes Restoration Initiative (GLRI) was initiated by the US Environmental Protection Agency (EPA) and Environment Canada (EC) in 2010. It provided funds primarily for restoration and clean-up projects across the Great Lakes, but assessments of the status and biological effects of both legacy and contaminants of emerging concern were also a significant component of GLRI. Tree swallows (Tachycineta bicolor), an aquatic passerine, are being used as a sentinel species because they nest in highly industrial and urban landscapes that are common in the Great Lakes but where few other waterbirds consistently nest. Their food habits, aquatic insects, provide an avenue for exposure from sediment contaminants to move up through aquatic and into terrestrial food chains. Data from four locations along the St. Louis River AOC will be presented and put in context with other AOCs. For polychlorinated biphenyls (PCBs), the mean exposure in tree swallow eggs on the St. Louis River as a whole, ranked 18th lowest out of 27 AOCs. On a site basis, the four sites ranked 24th (0.91 □g/g wet wt.), 44th (0.39 □g/g), 52nd (0.26 □g/g) and 62nd (0.15 □g/g) out of 69 sites. The latter three sites, Miller Creek, Hog Island, and Stryker Bay, were all at or below background for PCBs and similar to Wild Rice Lake, a nearby 'reference' site. Dioxin and furan exposure was similarly at or below background at all four sites as was mercury. Bioindicators of contaminant exposure were congruent with the exposure data and also indicated minimal physiological responses to contaminants. Data for pesticides, PFCs and PBDEs may also be presented.

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Ingestion of Microplastic Associated With Green Algae by Daphnia magna: Evidence of No Effect

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The concern of plastic pollution has recently grown at global scale. Recent studies have found that the Great Lakes region has the highest concentration of plastic material in any freshwater source. Concurrently, it is the largest source of freshwater in the world and therefore instigates a need to address the effect of these plastics. The present study determined the effects of microplastics (as defined as < 5mm in size) consumption in freshwater invertebrates, Daphnia magna. Daphnia magna was chronically exposed to fluorescent green polyethylene microspheres of size 63µm-75µm at concentrations of 25, 50, and 100 mg/L. Ingestion of microplastics and reproduction of D. magna were measured. The present study found that D. magna ingested significant amount of microplastics. The average concentrations of microplastics in the gut of D. magna were 26.26 and 76.75 pieces/organism at the lowest and highest water concentrations of microplastics, respectively. However, no statistically significant difference in reproductive rate was found for D. magna of control and exposure treatments. Evidence of increased algal production on microplastics compared to control is present. These results suggest that adsorbed algae on microplastics would provide an additional energy source for reproduction performance of D. magna. Organic pollutants can be absorbed by plastics and therefore can be carried to organisms when plastics are consumed. More research should be conducted to determine potential effect of adsorbed organic pollutants on plastics in the aquatic ecosystem.

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The Roles of Physicochemical Properties and Root Physiology In Predicting Plant Uptake of Pharmaceuticals And Personal Care Product Ingredients

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Crops irrigated with reclaimed wastewater or grown in biosolids-amended soils may take up pharmaceuticals and personal care product ingredients (PPCPs) through their roots. Risk assessment of consumption of contaminated crops requires predictions of uptake and bioaccumulation. However, predictive models used for neutral, nonpolar compounds are not sufficient to estimate PPCP uptake, as many PPCPs are polar/ionizable. We used uptake data from the literature to test two approaches to modeling accumulation of PPCPs in lettuce leaves based on physicochemical properties: simple empirical correlations based on octanol-water partitioning and a desirability model using multiple molecular descriptors. We found that molecular descriptors alone cannot adequately predict PPCP uptake and bioaccumulation into leaf tissues from hydroponic solutions or irrigated soil. It is likely that models will have to account for plant root structure and function to accurately predict root uptake and bioaccumulation in edible tissues. To begin to elucidate the uptake mechanisms of PPCPs, we are identifying uptake routes of selected PPCPs and investigating the role of energy availability in PPCP uptake. We have putatively identified PPCPs taken up via symplastic (through cells) and apoplastic (through the intercellular space) routes, and compounds with uptake affected by ATP availability. Understanding the role of plant root structure and function in uptake of PPCPs is important for developing the capacity to predict uptake and bioaccumulation of these compounds.

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Demonstration of Scalable Analytical Methods For The Screening of Algae Bloom Contaminated Surface Waters by UHPLC-TOFMS Equipped With A Novel And Automated Analyte Search Algorithm

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The advantages for mass spectrometry (MS) testing platforms have previously been reported for both small molecule screening and peptide applications versus traditional ELISA methods at ppb levels. of particular interest to this study is the emergence of MS as a means to monitor chemical markers of algae bloom contamination in surface waters. Time-of-flight (TOF) MS is ideal for this application, since researchers may not know the exact nature of contaminants for geographically isolated samples. Fast scanning TOFMS instruments offer identification by exact mass measurements and when paired with chromatographic separation tools (e.g. LC, UHPLC) will provide data that is more informing versus many orthogonal qualitative techniques. Preliminary method development was completed at the University of Central Florida (3 analytes) prior to technology transfer to the PerkinElmer Center of Excellence. The analyte panel was extended to 6 analytes, consistent with current trends in the field. Additional method optimization was required. Authentic samples of contaminated surface waters were obtained and prepared by Wayne State University (Detroit, MI) and shipped to PerkinElmer for analysis. To maximize the utility of this platform, a novel software package has been developed to allow for further interrogation of this sample set data without the need for further analytical method development or re-injection of the sample. The current template allows for >30 analytes. It is anticipated this data analysis template will prove a valuable tool in related environmental surveillance applications.

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Metabolomics: Dose Response in Cellular Assays

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The U.S. Environmental Protection Agency has approved four chemicals for the treatment of waterways that contain undesired species of fish. Potential new control chemicals are being identified by screening millions of compounds using structure-activity relationship models. These models, however, would be greatly improved by better understanding how fish metabolize those compounds. By combining cell culture and metabolomics, a powerful tool emerges to elevate the identification of new control chemicals. We combined the use of bighead carp fry cell line with metabolite profiling to describe the dose response to thiram. Thiram is a registered pesticide commonly used as a fungicide in the field or as a seed protectant and is known to be toxic to fish. Following exposure to 1.12, 1.87, 3.12, 5.2, 8.66, 14.43, and 24.08 ppm thiram, we identified 877 metabolites and 52 of those metabolites exhibited a dose response to thiram. Two of these metabolites have serotonin-like structures and may be indicators of stress. Combining cellular assays with metabolomic profiling may greatly enhance our ability to develop structure-activity relationship models to predict toxicity and identify new control chemicals.

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Bringing the Fathead Minnow into the Genomic Era

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The fathead minnow is a well-established ecotoxicological model organism that has been widely used for regulatory ecotoxicity testing and research for over a half century. While a large amount of molecular information has been gathered on the fathead minnow over the years, the lack of genomic sequence data has limited the utility of the fathead minnow for certain applications. To address this limitation, high-throughput Illumina sequencing technology was employed to sequence the fathead minnow genome. Approximately 100X coverage was achieved by sequencing several libraries of paired-end reads with differing genome insert sizes. Two draft genome assemblies were generated using the SOAPdenovo and String Graph Assembler (SGA) methods, respectively. When these were compared, the SOAPdenovo assembly had a higher scaffold N50 value of 60.4 kbp versus 15.4 kbp, and it also performed better in a Core Eukaryotic Genes Mapping Analysis (CEGMA), mapping 91% versus 67% of genes. As such, this assembly was selected for further development and annotation. The foundation for genome annotation was generated using AUGUSTUS, an ab initio method for gene prediction. A total of 43,345 potential coding sequences were predicted on the genome assembly. These predicted sequences were translated to peptides and queried in a BLAST search against all vertebrates, with 28,290 of these sequences corresponding to zebrafish peptides and 5,242 producing no significant alignments. Additional types of sequence data have also been layered onto the fathead minnow genome assembly to provide evidence of gene structures and other sequence elements. To this end, each of 240,000 fathead minnow expressed sequence tags (ESTs) and nearly 7,000 full-length zebrafish coding sequences (CDSs) were aligned to the genome assembly, with 73% and 38% creating successful alignments, respectively. A fathead minnow genome browser that provides accessible and visual integration of these various sequence datasets into a cohesive knowledge-base is being developed. . Completion of this work will provide a valuable resource for future ecotoxicology studies using the fathead minnow. The contents of this abstract neither constitute nor necessarily reflect official US EPA policy.

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Polybrominated Diphenyl Ethers (PBDES) In Fish From Wisconsin's Great Lakes And Inland Waters

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The Wisconsin Department of Natural Resources (WDNR) has been tracking bioaccumulating pollutants in fish that are consumed by wildlife, anglers, and anglers' families since the 1970s. Beginning in 2002, this effort has included monitoring levels of polybrominated biphenyl ethers (PBDEs) in Wisconsin sport fish from the Great Lakes and inland waters. The WDNR also has access to PBDE data from fish collected as part of the United States Environmental Protection Agency's 2003 National Lake Fish Tissue Study and 2010 National Coastal Condition Assessment Great Lakes Human Health Fish Tissue Study. Here, we summarize the concentrations of total PBDEs and proportions of PBDE congeners found in 26 fish species from 19 inland waters, Lake Michigan, and Lake Superior and explore the factors affecting PBDE accumulation in fish fillets. We found that PBDE contamination was spatially heterogeneous, and species with higher lipid content contained higher total PBDEs. Congener BDE-47 made up the highest proportion of total PBDEs in fillets of all species tested. Total PBDEs in fish sampled from the Great Lakes did not generally exhibit temporal variability, but proportions of congener types changed consistently through time, suggesting a possible shift in Great Lakes' PBDE origins. In terms of fish consumption advisories, total PBDE levels in most fish from most locations were not high enough to trigger exceptions to our statewide advice. Where more restrictive advice was warranted, the current advice due to PCB contamination was not superseded.

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Distinguishing between Bioaccumulation and Biomagnification of Triclosan using Macroinvertebrates

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Triclosan is an antibacterial chemical added to personal care products. Because large quantities of triclosan are produced and subsequently introduced into environmental systems, it is important to understand how it behaves in these systems, including its movement through food webs. Analytical methods were modified to extract triclosan and methyl-triclosan from water and biota samples. A bioaccumulation and biomagnification study was performed, using Chironomus riparius and Aeshnids (Odonata: Aeshnidae). C. riparius was exposed to a controlled amount of triclosan for 20 days and sampled periodically. Aeshnids, in separate aquaria, were fed triclosan-exposed C. riparius concurrently. They were sampled at the completion of the 20 days. Results indicate a decrease in concentration of triclosan in water, but no detectable concentrations in biota. Future work will focus on the improvement of the analytical methods and the transfer of this chemical into higher trophic levels.

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A Refined Multi-Site Model to Estimate the Toxicity of PAH-Contaminated Sediments at MGP Sites

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Many manufactured gas plant (MGP) sites have left a legacy of polycyclic aromatic hydrocarbon (PAH)–contaminated sediments in adjacent waterways. A refined multi-site sediment toxicity model was developed to predict the risk-based thresholds based on total PAH concentration in sediment. This refined model builds on earlier work completed on a smaller, less robust data set. The current model was based on data collected at four MGP sites in the Midwest. At one of these four sites, measurement of porewater PAH concentrations by solid-phase microextraction (SPME) methods was performed to evaluate the bioavailability of the PAHs. At each site, the toxicity of PAH-contaminated sediments to benthic invertebrates was evaluated using (1) laboratory-based sediment toxicity test results for Hyalella azteca, and (2) predictions of toxicity using the U.S. Environmental Protection Agency's equilibrium partitioning sediment benchmark (ESB) method. Site-specific, toxicity-based concentration thresholds for total PAHs (based on 13 or 34 analytes) varied considerably among the three sites (i.e., 45 mg/kg to 251 mg/kg total PAHs). To evaluate whether the variability in these threshold concentrations was a function of sediment characteristics, the data for all four sites were analyzed collectively. Total PAH concentrations were normalized based on total organic carbon (TOC) content, black carbon content, a combination of TOC and black carbon, or with the ESB method to evaluate the consistency of PAH thresholds. Receiver operating characteristic (ROC) curve analysis was used to determine whether each indicator (e.g., TOC-normalized total 13 PAH concentrations, ESB values, etc.) was a good predictor of sediment toxicity and to develop multi-site thresholds based on toxicity tests and ESB values. All indicators investigated were good predictors of survival, and very few adverse impacts on growth were observed. Thresholds estimated using the concentrations of total 13-PAH or total 34-PAH (and the normalized equivalents) were equally good indicators of toxicity. Thresholds predicted using the multi-site data set were compared with thresholds calculated for individual sites. This modeling effort demonstrated that ROC analysis of normalized data from multiple sites can be used to develop sediment toxicity thresholds that are often higher than traditional sediment quality benchmarks, but still are protective of aquatic organisms.

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A Survey: Demographics and the Rebound Effect in Vehicle Fuel Efficiency

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One of the pressing problems facing humanity in the 21st century is a changing climate, with the transportation sector emitting approximately a quarter of all emissions. Efforts to reduce emissions from transportation fall into two camps: market-based approaches of increasing fuel prices and improving fuel efficiency standards through legislation. Producing vehicles with improved efficiency allows consumers to travel a given distance using less fuel, leading to decreased emissions. An important consideration while quantifying the fuel savings from more efficient vehicles is to determine the rebound effect. The rebound effect occurs when an increase in efficiency of a good leads to increased consumption of that good. Accurately estimating the rebound effect in vehicles will show what percentage of the theoretical fuel savings is realized. This determination will offer insight into whether regulations pertaining to efficiency will lead to overall fuel savings. To gain understanding into the fuel efficiency rebound effect, consumer surveys were administered to gather data on driving habits and demographics. This study utilized the data to estimate the transportation rebound effect for various groups of participants. Groups were established based on demographic data, such as comparing rural and urban households and various income levels. The variations in the magnitudes of the rebound effect among the groups were analyzed to investigate factors that influence driving behavior and to what extent they affect fuel price elasticity. It is anticipated that comparing the rebound for households on different ends of demographic categories will reveal significant differences in their respective rebound effects. of particular interest was comparing rural and urban households and investigating the rebound differences. An important factor in this comparison is the availability of alternate modes of transportation, or lack thereof; when fuel prices increase, urban households can readily adopt alternate transportation or revert from it when fuel prices fall, whereas rural households may not have that option. These findings can be used to identify how different groups of drivers respond to improvements in fuel efficiency. Better understanding this behavior will provide discernment into the efficacy of using fuel efficiency mandates to reduce transportation emissions.

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Adsorption of Lamotrigine to Montmorillonite Clay

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Pharmaceutical compounds are often found in treated wastewater. Numerous agricultural regions utilize treated wastewater for irrigating crops. Consequently, these crops are exposed to pharmaceuticals, which have the potential to be taken up by plant roots. Currently, the ability to predict the extent to which pharmaceuticals can be taken up by plants is not well developed. Sorption to soil constituents is expected to exert strong control over the availability of pharmaceutical compounds to plant roots. Our preliminary studies have focused on the anticonvulsant drug lamotrigine, which has been previously found in treated wastewater and has a pKa of 5.7. We measured sorption of lamotrigine to montmorillonite clay equilibrated in KCl and plant nutrient solution over a range of pH values. We found that there was increased sorption at lower pH's when a higher percentage of lamotrigine was ionized. These results will be used to inform future studies on uptake of lamotrigine by plants grown in soil containing montmorillonite.

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Assessing the Conventional Calculated Method (CCM) of the Globally Harmonized System (GHS) for Acute Aquatic Toxicity Classifications of Chemical Mixtures Using 48-Hour LC50 Daphnia magna Test Results.

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The Globally Harmonized System of Classification and Labeling of Chemicals (GHS) represents an international approach to standardize the classification and communication of hazards for chemicals and chemical mixtures. Products used in the food and beverage (F&B) industries such as sanitizers, degreasers, and lubricants are examples of products that would be regulated under GHS. Such products are commonly disposed of in industrial wastewater effluent, which may or may not experience treatment prior to reaching an aquatic environment. For product stewardship, these products should be assessed and appropriately classified per GHS for aquatic toxicity. For this study, F&B commercial products were initially assigned GHS classifications for acute aquatic toxicity based on the conventional calculation method (CCM) as described by GHS. Twenty-four products with previously assigned CCM GHS classifications were selected for testing. The test used to evaluate these CCM classifications was a 48-hour LC50 acute toxicity assay using *Daphnia magna*. Aquatic invertebrate 48-hour LC50 values were determined and compared to the GHS classification criteria to assign a test-based classification. of the 24 products assessed, 15 had test-based classifications that aligned with the CCM classifications. For the remaining nine products, eight had test classifications that were less severe than the classification based on the CCM, while one product had a more severe classification after testing. of the eight products that had a downgrade in classifications based on testing, seven decreased by one GHS category and one decreased by two GHS categories, going from a Category 2 (>1 to ≤10 mg/L) to not classified (>100 mg/L) for acute aquatic toxicity. For the product with an upgrade in classification based on testing, it went from not being classified for acute aquatic toxicity (>100 mg/L) to a Category 3 (>10 to ≤100 mg/L). The results suggest that the CCM is reasonably accurate for GHS classification relative to aquatic invertebrate testing (15/24). In cases where the classifications from the CCM and aquatic invertebrate testing diverged, it generally appears the CCM results had a more conservative (8/9) classification. In conclusion, the CCM values were relatively accurate in assigning GHS classifications for the chemical mixture products evaluated using *Daphnia magna* and did not appear to result in appreciable under or over classification.

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Biological Effects of Septic Pollution on Larval Fathead Minnows, *Pimephales promelas*

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The potential of On-site Wastewater Treatment Systems (OWTSs) being a non-point source of contaminants into lake systems is an increasing concern for the survival of native fish species. OWTSs interact directly with groundwater and can leach contaminants out of their drain fields that include estrogens and pharmaceuticals. Since most lakes are down gradient of OWTSs, the contaminated groundwater easily enters the lake system through the hydrological process. The inflow of groundwater enters the shallow waters of lakes and interacts directly with the eggs of fish that are deposited in the sediment and the post-hatch larvae still residing in those waters. To explore the affects of these contaminants on the larvae, three study lakes were established that included the presence of two septic influenced and two reference sites per lake. Water analysis was performed to confirm the presence and absence of contaminants in their respective sites. Using a 21-day static renewal system, larvae were exposed to pore water pumped directly from the septic influenced and reference sites. In total, there were six septic influenced pore water treatments, six reference site pore water treatments, a blank well-water control, and an Estrone positive control. Once each 21-day exposure was complete, larvae from each treatment underwent behavioral testing that included the analysis of c-start behavior. For each larvae, the latency and escape response of the c-start were measured. For each treatment, the survival and growth of larvae were also measured. Although no significant differences between treatments were observed in this pilot study, preliminary chemistry analysis saw differences in the water samples of the septic and reference sites. We plan to expand the study for further research in the summers of 2016 and 2017.

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Assessing the Influence of Part Per Billion Variation of Natural Organic Carbon Levels on Cationic Polymer Acute Toxicity To *D. magna*

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Cationic polymers (CPs) are chemicals used widely for a variety of industrial and commercial purposes, for example, as floccing agents in water clarification. CPs generally have a low environmental concern due to the presence of organic carbon in surface water that greatly reduces their bioavailability, even at low mg/L levels. Although dissolved organic carbon can be as high as 50 mg/L in European surface waters, regulatory aquatic testing guidelines (OECD and EPA) limit total organic carbon (TOC) in clean dilution water to <2 mg/L to determine the baseline toxicity of chemical substances. However the amount, source and quality of the organic carbon in dilution water can vary considerably between testing laboratories. To better understand the influence of minute TOC differences as a potential sources of variation for clean water studies, we explored the impact of 0.25, 0.50 and 1 mg/L additions of TOC (as humic acid) in artificial water (Elendt M4 medium) on CP toxicity, all of which are considered by definition to be clean dilution water. We chose Daphnia magna to assess the acute toxicity of CPs with varying molecular weights and cationic charge density. Our results indicate that part per billion levels of TOC produced strong CP toxicity mitigation. These results suggest that background TOC in dilution water is a potential source of variation and should be considered when interpreting the results of aquatic toxicity studies. Therefore, we recommend that aquatic tests used to establish the baseline toxicity of cationic polymers should be standardized with added TOC in the dilution water at a minimally measurable amount (1 mg/L) to quench low level variability of background TOC and represent an environmentally realistic worst case exposure.

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Association of the Anticonvulsant Drug Lamotrigine With Dissolved Humic Acid

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Pharmaceutically active compounds (PhACs) enter agro-ecosystems via irrigation with effluent from wastewater treatment plants and amendment of soils with biosolids. Wastewater treatment processes do not eliminate PhACs from treated effluent leading to concerns about their uptake by plants and potential exposure to humans through dietary intake. Once introduced to the soil, the environmental mobility, bioavailability and uptake of PhACs by plants are expected to be influenced by their association with colloidal soil organic matter. We investigated the association of the anti-epileptic drug, lamotrigine (LTG) with dissolved Elliot soil humic acid (ESHA) as a function of pH and ionic strength. Lamotrigine possesses one ionizable moiety (Pka= 5.7), forming cationic LTG+ and neutral LTG0 depending on the solution pH. A relatively strong interaction between lamotrigine and humic acid was obtained, with the distribution coefficient of 10E4-10E4.6L/kgoc between pH 3 and 7 (ionic strength= 0.01 M), whereas almost no sorption occurred at pH 8 where LTG is primarily neutral. The association of LTG with ESHA depended strongly on pH, reaching a maximum near pH 5, which was consistent with association between the cationic LTG species and deprotonated sites in ESHA. Increasing ionic strength from 0.01 to 0.1 M resulted in approximately three-fold decreases in LTG-ESHA distribution coefficient, strongly supporting the contribution of cation exchange mechanism to sorption. Sorption data were well fitted by the Freundlich model, with Freundlich exponents of 0.78 and 0.81 at pH 4 and 7, respectively. The nonlinearity of the LTG-ESHA isotherms suggested LTG interacted with ESHA sites having a range of binding energies. Our results highlight the importance of considering lamotrigine speciation in environmental fate estimation and the strong influence of solution chemistry on LTG-ESHA binding.

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Bacterial Transformation of Engineered Nanomaterials Used In Electric Vehicle Energy Storage Devices

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Nanoscale lithium intercalation compounds (LICs) have attracted much attention as battery cathode materials for use in electric vehicles and portable electronics. Little information exists on the fate of LICs once released into the environment, which may occur upon disposal. Here, we have examined the dissolution of lithium nickel manganese cobalt oxide (NMC), a commonly used LIC, as a function of pH. We have further examined the influence of small organic acids and the bacterial siderophore, desferrioxamine B (DFO-β), on the dissolution of NMC. As pH increased less dissolved ions were detected in solution via inductively coupled plasma- atomic emission spectroscopy (ICP-AES). Incongruent dissolution of the ions was observed over time and as the pH changed so did the order in amounts of ions released. At a constant pH of 6, the organic acids citrate and oxalate, promoted dissolution in comparison to the water control. More ions were detected in the solutions treated with the citric acid over the oxalic acid for each of the metals with the exception of nickel. Furthermore, DFO-β promoted dissolution comparative to that of the oxalic acid. Dissolution of these materials produces locally high concentrations of toxic metals in bioavailable form.

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The Fate of Fragrance Chemicals from Wastewater Treatment Plants

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Synthetic musk chemicals are commonly used as fragrance additives in shampoos, detergents and other personal care products. Since many of these products get washed down the drain, musks have also been found in the aquatic environment. Studies have shown that musks bioaccumulate and biomagnify in aquatic food webs and have suggested that they can have negative impacts on ecological and human health, therefore it is important to understand their environmental fate. One major source of synthetic musk chemicals to the environment is wastewater treatment plant (WWTP) discharge. In order to study the abundance of these chemicals in southeast Wisconsin, samples of sludge and water effluent were taken from two local WWTPs. The four chemicals of interest in this study were galaxolide (HHCB), tonalide (AHTN), musk ketone (MK) and musk xylene (MX). Samples were collected, extracted, cleaned and prepared for GC/MS analysis based on EPA methods. HHCB was found in the highest concentrations both in sludge and effluent. It was followed by the other polycyclic musk, AHTN with the nitromusks (MX and MK) found in much lower concentrations. The information gained from this study could be used to assess risk and develop management plans for synthetic musk chemicals.

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Changes In Tyrosine Hydroxylase Expression in Zebrafish Embryos Resulting from Exposure to Bisphenol A (BPA)

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Bisphenol A (BPA) is a chemical widely used in food and drink packaging. Reports demonstrating the presence of BPA in the water and sediment of the Great Lakes, as well as BPA detection in freshwater organisms, suggest that BPA may have potentially profound ecological and environmental concerns. Confounding this problem is BPA's slow breakdown and deposition in soil, allowing for repeated exposure to aquatic organisms. A few reports have demonstrated alterations in motor behavior and dopamine levels in zebrafish; however, no studies have investigated the BPA's effects on dopamine cells in zebrafish embryos. We used quantitative polymerase chain reaction (qPCR) to measure the effects of BPA exposure on zebrafish embryos on the expression of tyrosine hydroxylase 1, which is the rate-limiting enzyme that produces dopamine in the central nervous system of zebrafish. Zebrafish embryos were exposed to 1, 5, 15 µM concentrations of BPA upon harvesting embryos and were prepared for qPCR five days post-fertilization. Two DNA primers specific to tyrosine hydroxylase 1 were used in the qPCR for quantification, and we compared the results of a known neurotoxin that depletes tyrosine hydroxylase in dopamine-containing cells, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). These initial results aim to establish zebrafish as a toxicology model and show the influence of sub-teratogenic levels of BPA on the developing dopamine system.

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***In Vitro* Assessment of Androgenic Activity of Water Reclamation Plant Effluents in Greater Chicago**

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The objective of this study was to examine whether water reclamation plants (WRPs) serve as an important source of androgenic contaminants in an urban waterway system (Chicago Area Waterways; CAWs). Grab water samples were collected in May 2009 from 38 locations spanning six watersheds. MDA-kb2 breast cancer cell line that naturally expresses androgen receptor was used to measure androgenic activity; activity was extrapolated from testosterone (T) standard curve using a non-linear regression. Upstream sites had significantly lower (Tukey test, $P<0.05$) androgenic activity (0.9 ± 1.4 ng/L T equivalents) than both downstream (DS; 3.9 ± 3.1 ng/L) and WRP (6.1 ± 3.7 ng/L) sites. Heavily urbanized and moderately urbanized sites exhibited similar androgenic activity. Observed spatial distribution of androgens (US < DS < WRP) indicates that WRPs are a source of androgens in CAW, but frequent detection of androgenic activity (43%) at US sites indicates that there may be additional sources of these contaminants (e.g., industrial, runoff). Nevertheless, unlike estrogenic activity, androgenic activity was largely associated with point sources of contamination (i.e. WRPs) and did not correlate with estrogenic activity. The urbanization intensity had no effect on the androgenic activity. This contrasts with estrogenic activity findings and provides further support for our hypothesis that non-point sources of pollution (e.g., runoff) are not an important source of androgens in the CAWs. Finally, general use water designation (which is not based on endocrine activity assessment) seemed to be protective as androgenic activity tended to be lower at sites with that designation.

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Effects of the Azole Fungicide Imazalil on the Fathead Minnow (Pimephales Promelas) Steroidogenesis Pathway

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Azole fungicides, used for both agriculture and human therapeutic applications, may disrupt endocrine function of aquatic life. Azole fungicides are designed to inhibit the fungal enzyme lanosterol 14 α-demethylase (cytochrome P450 [CYP] 51). However, they can also interact with and inhibit other CYPs, including those involved in steroid biosynthesis, through competitive binding to the heme moiety. High throughput screening in US EPA's Toxcast program identified imazalil as an endocrine active chemical capable of inhibiting mammalian aromatase (CYP19A1) and likely 17α-hydroxylase/17,20 lyase (CYP17A1). The present study confirmed this endocrine activity in fish. Exposure of reproductively mature female P. promelas to 100, 500 and 1580 µg/L imazalil for 24 h significantly reduced both testosterone (T) and 17β-estradiol (E2) production by ovary tissue collected from the fish. Likewise, plasma E2 concentrations in reproductively mature female P. promelas were significantly reduced following exposure to 80 and 250 µg imazalil/L, but not 2.5, 8, and 25 µg/L, for 24 h. The in vivo impacts of imazalil on fathead minnow steroid production were further supported by in vitro ovarian steroidogenesis assay in which ovary tissue exposed to the 200 µg imazalil/L in vitro synthesized significantly less E2 than tissue exposed to 0, 0.2, 2, or 20 µg imazalil/L. The present results support the use of an adverse outcome pathway linking aromatase inhibition to reproductive impairment in fish treatments and control, as a means to predict hazards, and potentially risk associated with exposure to this compound. The contents of this abstract neither represent, nor necessarily reflect official US EPA policy.

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Empirical Models for Quantifying the Role of Chemical Pollutants of Emerging Concern in Fish Biodiversity Loss in US

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Research investigating the regional and global impact of anthropic chemical species on biodiversity loss is scarce despite evidence of this effect. Some of these chemicals have become contaminants of emerging concern in aquatic ecosystems. This study contributes to sealing the gap in research by examining empirical relationships between the concentration trends of pesticides as emerging pollutants and freshwater fish species biodiversity decline. A mixed linear regression approach is used to evaluate the impact of multiple drivers of fish species biodiversity and pesticide mixture toxicity, ambient water temperature, total nutrient concentration as cumulative nitrogen and dissolved oxygen concentration over time in 6 regional US agro-urban streams. Pesticide mixture toxicity is calculated as Pesticide Toxicity Index (PTI) and fish species biodiversity is calculated as Shannon and Simpson Indexes of Diversity over time. The dominant model predictors, spatial and temporal sensitivity, as well as predictive capacity of the regression models developed are tested. Results show that the Northeast US Rivers ex- perienced a mean loss of 73% of miscellaneous native fish species from 1997 to 2014, as the residence-time pesticide toxicity (PTI) rose by 15% during the period. The species diversity loss for the US Midwest was 21% for a corresponding 8% increase in nutrient concentration. The Chattahoochee River (GA) showed a mean decrease in biodiversity of 28.2% in response to both pesticide toxicity and nutrient concentration with nutrient concentration having the more significant impact. The Santa Ana River (CA) indicated a mean biodiversity decrease of 43% with pesticide toxicity being more significant.

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Evaluating Chemical Tracers in Suburban Groundwater as Indicators of Nitrate-Nitrogen Sources

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Groundwater is an important resource. Approximately 30% of Wisconsin residents rely on private wells for their drinking water. Human activities, including agricultural practices and on-site wastewater discharge, can impact the water quality of these wells. Since 2000, almost 1 in 6 private wells tested in Portage County had nitrate-nitrogen concentrations that exceeded the groundwater standard. The objectives of this research were to: 1) develop an analytical method for a suite of indicators likely to occur in groundwater contaminated by human waste; 2) evaluate the relationship between groundwater nitrate-nitrogen and chemical indicators of on-site wastewater disposal and/or agricultural activities; and, 3) improve our understanding of groundwater movement and contaminant transport for use in subdivision planning and resource management. Eighteen private wells were sampled five times and analyzed to provide a temporal profile of nitrate and the tracers. Eight monitoring wells were sampled twice to examine variations with depth. The on-site waste indicator suite included artificial sweeteners, pharmaceuticals and personal care products. Metabolites of commonly applied pesticides were used to identify contamination due to agricultural practices. Ninety-six percent of private well samples with ≥ 3.0 mg N/L had at least one contaminant source indicator. The mixture of both agricultural and on-site waste compounds in the study wells is consistent with the importance of both to groundwater quality in the study area. Detection of agricultural contaminants in deeper wells is consistent with their distance from the study area. Longer groundwater travel distances lead to contaminants moving deeper in the aquifer. The on-site waste indicators were found in the shallower wells as expected for contaminant sources that are closer to the monitoring wells. The results of this research will help well users understand the sources of contamination, direct land management decisions, and select appropriate water treatment and remediation options.

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Evaluation of Traditional Soil Testing Methods to Estimate Lead Hazard In Soil

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Lead contaminated soils are nearly ubiquitous in urban areas as a result of leaded gasoline and lead-based paint use. While regulations banned leaded gasoline and lead-based paint decades ago in recognition of lead's deleterious health impacts, lead persists in soils long after deposition. Lead exists in various chemical "species" in soils with some species more easily absorbed (i.e. more bioavailable) than others. Phosphorus amendments are often recommended for lead contaminated soils because lead phosphates are among the least soluble of lead compounds. However, a rapid, inexpensive soil test that is able to detect changes in lead bioavailability after phosphorus amendments has yet to be identified. Our research explored the ability of four routine soil nutrient tests (DTPA, Mehlich 1, Mehlich 3, Bray P1) and two experimental assays (0.4 M glycine and 0.01 M calcium chloride) to estimate lead bioaccessibility as evidenced by a strong correlation with the Relative Bioaccessibile Leaching Procedure (RBALP) at pH 2.5. A silt loam soil was incubated with Pb(NO3)2 solution to attain to target lead concentrations of 0, 250, 500, 1000, and 2000 mg kg-1. After 12 days, a portion of these soils received H_3PO_4 and KCl at a rate of 10,000 mg P kg-1 soil and 3,000 mg Cl kg- 1 soil, respectively to induce lead phosphate formation. Treated soils also exhibited a reduced pH due to H_3PO_4 additions so they were raised to the original pH with 0.234 M NaOH via 7 wet/dry cycles. Results indicate that the DTPA and 0.4 M glycine methods were most sensitive to P amendments as indicated by a 79.0% and 75.1% average reduction in extracted lead after P additions. Mehlich 3 extracted lead was most strongly correlated to the RBALP extracted lead at pH 2.5 (r^2= 0.93). Our findings suggest the DTPA and 0.4 M glycine extractions are capable of detecting P induced reductions in lead bioaccessibility and Mehlich 3 is a promising surrogate for the RBALP (pH 2.5) as a tool to determine lead bioaccessibility. These observations are significant because they illustrate that simple nutrient extractions may help make accurate lead bioaccessibility information more accessible to the public.

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Evaluation of Whole-Mount In Situ Hybridization as a Tool for Pathway-Based Toxicological Research in Early-Life Stage Fathead Minnows

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Early-life stage fish can be more sensitive to chemical exposure than mature, adult fish. Therefore, defining adverse outcome pathways (AOPs) relevant to early-life stages is critical for linking perturbations of key events during fish development to potential adverse outcomes of chemical exposure. To determine chemical effects and/or mechanisms of action in exposed fish embryos and larvae, whole mount in situ hybridization (WISH) paired with quantitative polymerase chain reaction (QPCR) assays hold excellent promise. While WISH has frequently been used in zebrafish (Danio rerio) early-life stage developmental work, this technology has not previously been applied to fathead minnows (Pimephales promelas), another well-established laboratory small fish model. In the present study, WISH was implemented in fathead minnow embryos and larvae as a tool to aid in the development of AOPs associated with early-life stages. As a proof of concept, fathead minnow embryos were exposed to the known estrogen receptor agonist, estrone (0, 18, and 1800 ng/L), for 3 and 6 days in a solvent-free, flow-through test system. Relative transcript abundance of three estrogen-responsive genes, estrogen receptor-alpha (esr1), vitellogenin (vtg), and cytochrome P450-aromatase B (cyp19b) was examined in pooled whole embryos using QPCR, and spatial distribution of significantly up-regulated gene transcripts was further examined using WISH in individual fish. After 3 d of exposure to estrone, relative transcript abundance of esr1 and cyp19b was significantly up-regulated, while vtg mRNA expression was not significantly affected. Transcripts for all three genes were significantly up-regulated after the 6 d exposure to 1800 ng estrone/L. Subsequently, WISH assays revealed spatial distribution of esr1 and vtg in the liver region, suggesting that estrone is activating estrogen receptors in the liver of exposed embryos after 6 d of exposure. Using WISH (as a complement to QPCR) to determine which specific tissues are targeted during chemical exposure has potential to lend insight relative to those biological pathways perturbed by chemicals of interest. Consequently, WISH may be particularly useful for further investigation of AOP development in fathead minnows, notably identification of tissue-specific alterations in key molecular initiating events (e.g., ER activation in the liver in this study) that may lead to subsequent effects on early-life stage development.

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Evidence of Lampricide Photodegradation During Field Applications to Tributaries of the Great Lakes

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The lampricide 3-triflouromethyl-4-nitrophenol (TFM) has been used to kill the invasive sea lamprey in tributaries of the Great Lakes since the 1950's. In contrast to legacy contaminants and many chemicals of emerging concern, TFM is directly added to the Great Lakes ecosystem. Despite its widespread and intentional use, the fate of TFM in the environment is still poorly understood. We performed laboratory experiments to assess the susceptibility of TFM to photochemical degradation and conducted two field campaigns to quantify TFM photolysis during lampricide applications in 2014 and 2015. The laboratory experiments indicate that TFM undergoes pH-dependent direct photolysis with a quantum yield of 1.22 x 10-4 at pH 7. Approximately 30% of TFM undergoes dehalogenation to form gentisic acid under these conditions. Samples were collected during a TFM application in the Manistique River, a major 70-mile tributary to Lake Michigan, in 2014. Concentrations of TFM and gentisic acid were 7.38 ± 1.92 µM and 1.94 ± 0.55 µM, respectively, providing clear evidence of TFM photolysis in this large river system. An extensive field campaign was conducted in two smaller tributaries to Lake Superior (i.e., Sullivan Creek and Carpenter Creek) in 2015. By comparing TFM concentrations with results from a time of passage study in both creeks, our results indicate minimal loss of TFM to photodegradation as the lampricide traveled through the approximately one-mile tributaries. Therefore, TFM can be expected to undergo substantial photolysis in larger river systems, but the lampricide may enter the Great Lakes with minimal degradation when it is applied to smaller tributaries. Photolysis of TFM within the Great Lakes is expected to be minimal due to the limited extent of the photic zone. These results have implications for the impacts of TFM on the Great Lakes ecosystem.

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Exposure to two 2,4-D Herbicide Formulations Decreased Larval Survivorship In Fathead Minnows (Pimephales promelas)

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Exposure to two 2,4-D herbicide formulations decreased larval survivorship in fathead minnows (Pimpephales promelas) Dehnert*, G., M.B. Freitas*, Z.A. DeQuattro, T.P. Barry, and W.H. Karasov Commercial formulations of the aquatic herbicide 2,4-dichlorophenxyacetic acid, dimethylamine salt (2,4-D) are widely used to control weeds in lakes managed by state and federal resource management agencies in the US. We investigated the effects of two 2,4-D formulations, Weedestroy® AM40 and DMA® 4 IVM, on the survival of fathead minnow (Pimpephales promelas) larvae in two separate studies. In the first study, fathead minnows were continuously exposed to graded doses (0, 0.05, 0.50, and 2.00 ppm) of two commercial formulations of 2,4-D immediately after fertilization until 30 days post-hatch, in a flow through system. Weedestroy® AM40 decreased larval survivorship at 0.50, and 2.00 ppm ($p < 0.05$) while DMA® 4 IVM decreased larval survivorship at 0.50 ppm ($p < 0.05$). In the second study, groups of reproductively mature fathead minnows (2 females and 1 male in 7L aquaria) were exposed to graded doses (0, 0.05, 0.50, and 2.00 ppm) of Weedestroy® AM40 for 28 days; their offspring were then subsequently exposed to the same dosages of 2,4-D as their parents, from fertilization until 30 days post-hatch. Weedestroy® AM40 decreased larval survivorship in fish exposed to 0.05, 0.50, and 2.00 ppm ($p < 0.05$).). The results from these experiments combined with earlier data published by our group, which was similar in design to the second study but on DMA® 4 IVM, illustrates that commercial 2,4-D formulations can decrease larval survivorship of larval fathead minnows, and suggests that parental exposure may increase larval sensitivity to the herbicide. These data indicate that caution needs to be exercised when using commercial 2,4-D formulations to manage lake weed populations as it may limit the survival of larval offspring. Funded by Wisconsin Department of Natural Resources and Department of Forest and Wildlife Ecology, University of Wisconsin-Madison.

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Examining the Demographic Effects of Coal Fly Ash Exposure Routes in *Daphnia magna* and *Daphnia pulex*

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Coal fly ash is a by-product of burning coal for electricity which contains potentially toxic elements such as arsenic, mercury, barium, cadmium, chromium, lead, selenium, and silver. To assess the demographic effects of coal ash exposures on primary consumers in aquatic systems, two species of *Daphnia* were exposed to coal fly ash from both water and algal foods. After 29 days of exposure, cumulative reproduction in *Daphnia pulex* in the water only exposures decreased by 9 neonates compared to controls. Additionally, *Daphnia magna* mortality in all of the water only exposures indicated that acute toxicity may be due to the heterogeneous nature of coal fly ash. Exposing young *Daphnia* to coal fly ash negatively effects the primary consumers population which decreases the energy transferred to secondary and tertiary consumers in aquatic ecosystems.

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Impacts of Early Exposure To Triclosan on Growth, Maturation, and Reproduction in Zebrafish

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Triclosan (TCS) is an antimicrobial agent found in many personal care products. It is one of the most frequently found personal care products in surface waters, and has been found to bioaccumulate in aquatic organisms. TCS is an endocrine disruptor in fish, and at high concentrations can alter sex differentiation and reproductive capacity. Here we examine the effects of exposure to environmentally relevant concentrations of TCS during larval development on growth, maturation, and reproductive capacity in zebrafish. Zebrafish were exposed to vehicle control, 0.4, 4, or 40 □g TCS/L from 21-35 days post fertilization via static waterborne exposure with daily renewal (50%). Fish were raised in TCS-free water until they reached adulthood, when we evaluated impacts on reproduction. While survival was not impacted, growth was slightly reduced, and we found that TCS-exposed fish matured slower. Preliminary findings suggest that sex ratios are altered, as well as reproductive success. The results of our study will aid in understanding conflicting hypotheses regarding the mechanisms that TCS disrupts the endocrine system, and help us to better understand the potential risks of TCS to wild fish populations.

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The Effects of Chlorinated Solvents on Tyrosine Hydroxylase 1 Expression In Zebrafish Embryos Using Quantitative Polymerase Chain Reaction (qPCR)

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Chlorinated solvents, such as tetrachloroethylene (PCE) and trichloroethylene (TCE), are common industrial solvents and environmental contaminants that have been linked to Parkinson's disease. Locally, PCE vapors are problematic on Madison's east side due to groundwater and soil contamination with PCE by the Kipp Corporation. Parkinson's disease is caused by the degeneration of brain cells containing the neurotransmitter dopamine. Despite the epidemiological studies that indicate a connection between these chlorinated solvents and Parkinson's disease, dopamine depletion studies caused by these solvents are lacking, and no animal model for solvent-induced dopamine loss has emerged. Using zebrafish embryos, we sought to demonstrate that PCE and TCE deplete dopamine-containing cells by measuring tyrosine hydroxylase, the rate-limiting enzyme that produces dopamine. In order to test the effects of chlorinated solvents on dopamine-containing cells, we used quantitative polymerase chain reaction (qPCR) to measure the expression of the enzyme that produces dopamine in the zebrafish central nervous system, tyrosine hydroxylase 1, after exposure to PCE and TCE. Zebrafish embryos were exposed to 10 mM PCE and TCE four days after fertilization for 48 hours, and then prepared for qPCR using two DNA primers specific to tyrosine hydroxylase 1. The effects of these solvents on zebrafish expression of tyrosine hydroxylase 1 are compared an 800 μM exposure to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) because MPTP is an established neurotoxin that depletes dopamine-cells and leads to a loss of tyrosine hydroxylase. The initial results suggest a trend toward a decrease in tyrosine hydroxylase 1 expression after PCE and MPTP exposure, but more replicates are needed. Replications of these experiments are currently underway. These studies seek to demonstrate that chlorinated solvents deplete tyrosine hydroxylase in dopamine-containing neurons and further validate using zebrafish embryos to test for neurodegenerative effects of environmental toxins.

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Interaction of Nanoparticles with Gram-Positive Bacterial Surfaces

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Production and use of engineered nanomaterials has rapidly increased since the early 2000s. Manufacturing of nanoparticles is undoubtedly advancing fields like medicine and energy, but at present, we lack knowledge of unintended consequences of nanomaterials entering the environment. To study the extent of these consequences, we must understand nanomaterial interaction with a variety of biological systems, including bacteria. Biological systems derive their high functionality from structural complexity at the nanoscale, and one of the primary modes of adverse nanomaterial interaction requires contact at the cellular surface. Gram-positive bacteria are found in terrestrial and marine sediments and are important in biogeochemical cycling in these environments. Our objective is to determine the critical chemical components of the cell surface that govern nanoparticle interactions. We used diamond nanoparticles functionalized with positively charged polymer poly(allylamine hydrochloride) as a model system for a nanoparticle with an inert core and charged surface. We are using complementary spectroscopic, imaging, and in situ monitoring techniques to study the interaction of these nanoparticles with Gram-positive cell surfaces. Our preliminary solid state NMR results suggest that positively charged diamond nanoparticles associate with wall teichoic acids, anionic copolymers of the Gram-positive bacterial cell wall. We expect that our results will ultimately inform the assessment of risks posed by nanomaterials released to the environment, as well as nanoparticle manufacturing to modulate interactions with Gram-positive bacteria.

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Tree Swallows as Indicators of Sediment Contamination

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A basic premise when using contaminant concentrations in nesting tree swallows (*Tachycineta bicolor*) is that tissue concentrations are sediment based and of local origin. Studies have documented that tree swallow diet is mainly composed of aquatic insects and an earlier study based on limited sample size from five different studies described a significant, positive association between polychlorinated biphenyl (PCB) concentrations in sediment and tree swallow eggs and nestlings. We add to this assessment by demonstrating a significant relationship between PCB concentrations in sediment and PCB concentrations in eggs, nestling diet, and nestling carcasses across 26 Great Lakes Areas of Concern (AOC). Local contamination is demonstrated by differences in contaminant concentrations between nearby nesting sites. For example, PCB concentrations increased downriver on the Ottawa River, OH from Tyler Landfill (mean = 308 ng/g) to Dura Landfill (470 ng/g) to Hoffman Landfill (941 ng/g). Concentrations of PCBs were significantly higher at Hoffman than Tyler Landfill, only three km downriver. In another instance, perfluorinated compound concentrations in nestling plasma were significantly higher at Ryerson (188 mg/ml) than Hartshorn Marina (40 ng/ml) two sites 3.4 km apart in Muskegon Lake, MI AOC. Even though tree swallows are migratory and could conceivably accumulate some contaminants away from the area of collection, the strong association between PCBs in eggs and sediment demonstrates that contaminants in tree swallow eggs are of local origin. These results corroborate other data that suggest that tree swallows are 'interest breeders' relying on nutrient sources collected locally on the breeding grounds for egg formation. Female tree swallows base egg-laying decisions, especially timing, on short-term income from recent foraging bouts rather than on long-term somatic stores.

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Population Effects of Coal Fly Ash-Exposed Chlamydomonas reinhardtii Fed to Daphnia Magna at Low and High Food Concentrations

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Coal fly ash is the powdery by-product of burning coal to generate electricity in power plants. *Daphnia magna* are primary consumers in aquatic ecosystems commonly used in toxicity testing. The constituents of coal fly ash have been shown to cause adverse health effects, but the population-level consequences of coal fly ash from the Tennessee Valley Authority Kingston coal ash spill on aquatic consumers are unknown. In the 30-day experiment presented here, we fed *D. magna* an algal food that was exposed to coal fly ash to study population level effects due to the ash. A high food, no fly ash control treatment produced *Daphnia* averaging 4.00 mm in length and producing 54.25 neonates, compared to 3.79 mm and 44.25 neonates in the high food, high ash treatment, respectively. This influence on growth and cumulative reproduction suggests that coal fly ash from the Kingston spill will affect the *D. magna* species present within those aquatic systems and potentially organisms further up the food web.

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Defining Great Lakes Tributary Representative Mixtures For Chemical Exposure Experiments

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A collaborative study conducted by the U.S. Fish and Wildlife Service, U.S. Geological Survey, St. Cloud State University, and the University of St. Thomas is characterizing and evaluating the risks that contaminants of emerging concern (CECs) are posing to fish and wildlife resources in the Great Lakes Basin. Water and sediment samples were collected from over 200 sites across 25 Great Lakes tributaries from 2010 to 2014 and analyzed for a broad suite of CECs including hormones, pharmaceuticals, alkylphenols, pesticides, and flame retardants. A multi-faceted approach was used to identify commonly occurring chemical mixtures representative of Great Lakes tributaries, which are being used in a multi-generational laboratory exposure to evaluate multiple levels of biological response in fathead minnows. The approach included the use of multivariate statistics, chemical mixtures software, co-collected biological response data, and documented biological effects data from peer-reviewed literature. Results from multivariate statistical tests indicated chemical patterns reflective of land use and targeted point sources. Sites with the highest pesticide concentrations were those with the greatest agricultural influence. Sites with the most complex mixtures of contaminants were associated with sample sites downstream of urban point sources (e.g. wastewater treatment facilities and combined sewer outfalls). These mixtures were often dominated by pharmaceuticals, hormones, and flame retardants. A suite of chemicals were chosen that represent each of the different influences (agriculture or urban) and were evaluated using a chemical mixture software package that identified the sample sites where these representative mixtures were detected. The data from these sample sites were then used to determine chemical concentrations to be used in the multi-generational exposures, ensuring that the exposures would be represent these Great Lakes tributaries. Results from the multi-generational exposures will improve understanding of how mixtures of CECs, as measured in the Great Lakes Basin, impact fish resources. This information will help guide future resource management and restoration activities in the Great Lakes.

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Chronic Effects of Lead to Topsmelt Fish (*Atherinops affinis*); SSD Development, Influence of Salinity,and Influence of Organism Age

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Lead (Pb) occurs in the environment as a consequence of both natural and anthropogenic processes, with mining and smelting, coal burning, and cement manufacturing contributing most of the Pb contamination of aquatic environments. The present study to determine the chronic toxicity of Pb to topsmelt (*Atherinops affinis*) at two different salinity and organism ages in support of development of species sensitivity distribution (SSD) for ecological risk assessment and setting water quality criteria for Pb.The study was conducted for 28 days in a water flow-through testing system. Survival, standard length, dry weight, and Pb tissue concentration were measured. Measured dissolved Pb concentrations were used to calculate lethal effect concentrations (LC), and effective concentrations (EC), and bioconcentration factor (BCF). In general, increasing salinity and organism age decreased Pb toxicity. The 28-d LC50 values for larval fish at 14 and 28 ppt salinity were 15.14 and 79.84 µg/L dissolved Pb, respectfully. For juvenile fish and 28 ppt salinity, the 28-d LC50 was 167.6 µg/L dissolved Pb. Using standard length, the EC10 values for larval fish was 16.63 and 82.30 µg/L dissolved Pb at low and high salinity, respectively. The dry weight EC25 for low and high salinity were 15.62 and 46.85 µg/L dissolved Pb, respectively. The BCF was higher (2.00) in test with lower salinity (0.73). This is likely due to competition of salt ions with Pb for binding at the biotic ligand. These results will be used for the Pb chronic marine SSD and marine Pb water quality criteria.

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Enhancement of Microplastics on Endosulfan Sulfate Uptake In Earthworm (*Lumbricus terrestris*)

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Plastic pollution has been a concern by environmental scientists. Research on potential effect of microplastics has been conducted but at initial phase and more focused on marine ecosystem. Research has shown that organic contaminants can be adsorbed on plastics and carried into organisms when plastics are consumed. The present study examined the effect of microplastics on the accumulation of endosulfan sulfate in Lumbricus terrestris. Organisms were exposed to control soil and endosulfan sulfate contaminated soil that was spiked with different microplastics concentrations for 28 days based on the standard bioassay. Earthworm were collected on days 0, 7, 14, 21 and 28 for andosulfan sulfate analyses. Results of the present study showed that microplastics enhanced endolsulfan sulfate accumulation in the earthworms. Tissue concentration of endolsulfan sulfate increased over time and was higher in treatments with higher microplastics concentrations, such as 12 ng/g compared to 40 ng/g, respectively. More microplastics were also found in worms that were exposed to higher concentration of microplastics. Visually, earthworms in treatments with higher concentration of microplastics and endolsulfan sulfate did not perform well and consumed less food. To our knowledge this is the first study on the effect of microplastics on bioaccumulation of endosulfan sulfate in earthworms.

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Characteristics of Wisconsin Male Anglers over 50 Related to Fish Consumption and Advisories

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The Environmental Protection Agency Great Lakes Restoration Initiative supported evaluation of Wisconsin's fish consumption advisory program using a web based survey of male Wisconsin anglers over the age of 50 to test advisory awareness, comprehension, and effectiveness. A total of 3740 men completed the online survey. Comprehension of guideline content was relatively high, although knowledge gaps were identified. In general, Wisconsin's consumption guidelines do not appear to discourage men from eating the fish they catch; rather, the most common behavioral changes included modifying the species eaten or the water body source of their meals. The second part of this study recruited participants to provide blood and hair samples and complete a detailed (paper) questionnaire. Biological samples were used to assess levels of PCBs, PBDEs, PFCs (blood), and mercury (hair and blood). Participants' fish consumption included mostly locally caught fish and they had somewhat higher mercury levels compared with the US general population. Consumption of fish was associated with higher levels of each of the contaminants with the exception of PBDE. The effect of supplement use was much greater than that of fish consumption on the participants' nutrient levels, although consumption of some fish from specific locations was associated with higher levels of vitamin D.

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Method Refinements for the Midge Life-cycle, *Chironomus dilutus* Test

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Larval stages of non-biting midges can be found in almost any freshwater ecosystem, and one of the commonly tested midges is *Chironomus dilutus* (Chironomidae, Diptera) which is used for toxicity testing and ecological risk assessment of freshwater contaminants. USEPA, ASTM, Environment Canada, and OECD have published standard methods describing the sediment toxicity tests with various species of midges. USEPA/ASTM's current guidelines (2000) provide procedures for a midge life-cycle test that begins with newly hatched *C. dilutus* larva (~1-4 h after hatching free from egg mass). However, since the method was first published, many laboratories have observed wide variability in control survival across tests and many tests have not met the ≥70% control survival requirement. We are pursuing experiments to better understand the variability in control survival, and to explore techniques that will improve control performance. One line of experimentation is to compare performance of 1-4 h old larvae and 3-4-day old larvae in natural sediment or quartz sand. Previous studies have indicated a difference in sensitivity between <1-h old and 8-d old larvae for certain toxicants; we are interested in whether use of 4-d-old midge might improve typical control survival while retaining some of the greater toxicant sensitivity observed with younger organisms. *This abstract does not necessarily reflect US EPA policy.*

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