CPRC SETAC 2018 Annual Spring Meeting
University of Mary Washington,
Jepson Alumni Executive Center,
Fredericksburg, VA

April 22 & 23, 2018

Program & Abstracts

Additional information is available at:
CPRC SETAC 2018 Annual Spring Meeting

EARLY REGISTRATION ENDS APRIL 14TH!
REGISTER HERE
Password: CPRCSETAC2018
Welcome CPRC Members!

The 2018 CPRC SETAC Spring Meeting celebrates the 35 years of this chapter since its establishment in 1983. I am pleased to welcome you to the historic city of Fredericksburg and the University of Mary Washington (my alma mater) as the location and venue, respectively, for this year’s meeting. CPRC’s Events Planning Committee plans the annual spring meeting each year to encourage students and professionals in industry, academia and government agencies of the Chesapeake-Potomac Region to network and discuss topics related to environmental toxicology and chemistry. The program for the 2018 Spring Meeting features presentations on methodologies for environmental and toxicological analyses, ecotoxicology and testing challenges, remediation, environmental contaminants, hazard/risk assessments, and more.

Similar to previous spring meetings, a short course will be offered on Sunday afternoon. This year’s course “Fundamentals of Ecological Risk Assessment” will be given by Tim Iannuzzi, Senior Vice President/Principal Scientist at Arcadis and current CPRC SETAC board member. An outline of the short course is available online and will cover topics such as environmental risk assessment framework, effects assessment, risk characterization, environmental data, and much more! Following Sunday’s short course, we welcome all to gather in downtown Fredericksburg for an informal social event (location TBD). This event is a great opportunity to meet our newly elected board members and executive officers.

Monday’s technical meeting features over 20 scientific presentations, a lunch-time student mentoring event, and cash awards for Best Student Presentations (platform and posters). After morning registration, the first session will commence with opening remarks followed by student and professional platform presentations and a networking/poster social. The student-mentoring activity will be held at lunch time and is a wonderful opportunity for students to interact with professionals from diverse backgrounds and to ask questions and seek career advice (sign up as a student mentee or mentor during registration!). Following lunch, Jennifer Flippin, CPRC Vice President will introduce the afternoon session and Alex MacLeod will update those in attendance about SETAC North America and NASAC activities. At the conclusion of the afternoon general session, the keynote address entitled “35 Year Historical Overview of CPRC-SETAC and the Field of Ecotoxicology” will be given by Dr. Barnett Rattner, a distinguished scientist at the Patuxent Wildlife Research Center of the Department of the Interior, Adjunct Professor at the University of Maryland, and a Charter Member, Fellow and Past-President of the SETAC-NA. Finally, the meeting will conclude by announcing the student award winners for best platform and poster presentations and the raffle prize winner.

I look forward to seeing you in Fredericksburg, April 22-23rd to celebrate CPRC’s 35 years!

All the best,

Ben Burruss
CPRC SETAC President
Sunday’s Short Course

“Fundamentals of Ecological Risk Assessment”,
by Timothy Iannuzzi, Principal Scientist at Arcadis

Time: Sunday, April 22, 2018 1:30 - 5:00 PM

Place: University of Mary Washington,
Jepson Alumni Executive Center,
1119 Hanover Street, Fredericksburg, VA

Tim Iannuzzi is an ecologist/biologist with 30 years of experience in the field of Ecological Risk and Impact Assessment. He is presently a Principle Scientist and Leader in the Ecology, Risk Assessment and Natural Resources Damage Assessment (NRDA) practices within Arcadis, and international engineering and sciences consulting company. His management experience ranges from screening-level environmental assessments to complex, risk-based investigations of ecological and human health, NRDA, environmental impact evaluations, and ecological restoration.

Mr. Iannuzzi also teaches at the University of Maryland in ecological risk and impact assessment and is continuously involved in research into key areas related to estuarine and freshwater ecology, risk/impact assessment, NRDA, bioaccumulation modeling/assessments, and historical ecology. His publications include more than 35 technical papers in scientific journals, two book chapters, and is senior author on a book titled A Common Tragedy, History of an Urban River (Amherst Press, 2002).

This course will provide an introduction into the science and practice of ecological risk assessment. This course will focus on the application of USEPA’s Ecological Risk Assessment (ERA) Framework and Guidelines for the evaluation of chemical contamination in the environment. Application to non-chemical stressors will also be discussed. Participants will be exposed to an array of interrelated disciplines and concepts that are applied in the practice of risk assessment and weight-of-evidence impact assessment. These include basic toxicology, systems ecology, population biology, fate and transport of chemicals in the environment, empirical modeling, environmental data issues, and regulatory policy.

And, after Sunday’s short course come join CPRC for an informal social of food and drinks in downtown Fredericksburg.
Mondays Keynote Speaker

Dr. Barnett Rattner, will be presenting this year’s keynote address entitled “35 Year Historical Overview of CPRC-SETAC and the Field of Ecotoxicology”. Dr. Barnett Rattner is a scientist at the Patuxent Wildlife Research Center of the Department of the Interior and an Adjunct Professor at the University of Maryland. He received his doctorate in Zoology in 1977 from the University of Maryland, and following a one year National Research Council Postdoctoral Associateship at the Naval Medical Research Institute, he joined the U.S. Fish and Wildlife Service Patuxent Wildlife Research Center. He conducts research of regional, national and international scope that is of high priority to the Federal Government. This entails hypothesis-driven laboratory and field investigations, risk assessments and scholarly evaluations of the hazard and toxicity of legacy (e.g., industrial compounds, metals, and pesticides) and contemporary pollutants to wildlife and the environment. The focus is on exposure and adverse effects of emerging contaminants (e.g., flame retardants, pharmaceuticals), comparative toxicology, non-target secondary poisoning associated with rodenticides, alternative testing methods, and screening-level risk assessments. He has nearly 40 years of professional experience, published over 125 peer-reviewed articles and book chapters, co-edited three reference texts, and serves on several journal editorial boards and statutory interagency government panels. He is a Charter Member, Fellow and Past-President of the Society of Environmental Toxicology and Chemistry.

Support CPRC Student travel awards!

Support CPRC student travel award program by ordering a CPRC 35th Anniversary t-shirt. All net proceeds will go towards CPRC student travel award program. Order your t-shirt directly online – click here to order!!
<table>
<thead>
<tr>
<th>Time</th>
<th>Meeting Program - Monday April 23, 2018</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:00 – 8:30 AM</td>
<td>Set-up - Posters and Sponsors</td>
</tr>
<tr>
<td>8:30 – 9:15 AM</td>
<td><strong>Registration Opens / Breakfast / Networking &amp; Poster Social</strong></td>
</tr>
<tr>
<td>9:15 – 9:30 AM</td>
<td><strong>Welcome and Opening Remarks</strong> – Darci Ferrer, CPRC President Ben Burruss, CPRC Vice President</td>
</tr>
<tr>
<td>9:30 – 9:45 AM</td>
<td>*Mandar Bokare, UMBC - Quantification of water-air transfer rates for PCB’s and OCP’s in the Anacostia River using a passive sampling approach</td>
</tr>
<tr>
<td>9:45 – 10:00 AM</td>
<td>*R.M. Harrison, UMD - Survival and biochemical health indicators of Elliptio complanata deployed in Anacostia River tributaries for monitoring of persistent organic contaminants</td>
</tr>
<tr>
<td>10:00 – 10:15 AM</td>
<td>*Trevor Needham, UMBC - Microbial Interaction with Carbonaceous Material and the Implications for Environmental Remediation</td>
</tr>
<tr>
<td>10:15 – 10:30 AM</td>
<td>*Patrick Raya, GWU - Processing EPA’s ToxCast andTox21 High Throughput Screening Data for Hazard Assessment</td>
</tr>
<tr>
<td>10:30 – 11:15 AM</td>
<td><strong>Refreshment Break and Poster Social</strong></td>
</tr>
<tr>
<td>11:15 – 11:30 PM</td>
<td>Hans Plugge, Verisk 3E - Enterprise-wide Risk and Hazard Assessment</td>
</tr>
<tr>
<td>11:30 – 11:45 PM</td>
<td>Traci Clymer, GWU - Computational redesign of organophosphate flame retardants and pesticides for improved safety and efficacy</td>
</tr>
<tr>
<td>11:45 – 12:00 PM</td>
<td>Susan Thomas, EAG Laboratories - Establishing equilibration of chemical concentration in pore water and sediments prior to conducting sediment toxicity tests</td>
</tr>
<tr>
<td>12:00 - 12:15 PM</td>
<td>James Occhialini, Alpha Analytical - Potential for PFAS Cross-Contamination from Sampling Equipment and Associated Products</td>
</tr>
<tr>
<td>12:15 – 12:30 PM</td>
<td><strong>Hank Krueger, EAG Laboratories - Challenges in Conducting Tier 1 Honey Bee Tests</strong></td>
</tr>
<tr>
<td>12:30 – 1:45 PM</td>
<td>Lunch &amp; Mentor/Mentee Tables</td>
</tr>
<tr>
<td>1:45 – 2:00 PM</td>
<td>Afternoon Session – Jennifer Flippin, CPRC Vice President (incoming)</td>
</tr>
<tr>
<td>2:00 – 2:15 PM</td>
<td><strong>SETAC-NA Update</strong> – Alex MacLeod, UMD</td>
</tr>
<tr>
<td>2:15 – 2:30 PM</td>
<td>D.S. Brougher, EAG Laboratories - Expanding the Number of Freshwater Invertebrates used in Acute Toxicity Tests for the Registration of Chemicals</td>
</tr>
<tr>
<td>2:30 – 2:45 PM</td>
<td>Matthew Fiala, Texas Southern University - Chemical mass balance source apportionment of trace metals in road dust</td>
</tr>
<tr>
<td>2:45 – 3:00 PM</td>
<td>Nathalie Lombard, UMBC - Persistent organic pollutant monitoring in the Anacostia watershed using passive sampling and uptake in a freshwater mussel</td>
</tr>
<tr>
<td>3:00 – 3:45 PM</td>
<td>Refreshment Break and Poster Social</td>
</tr>
<tr>
<td>3:45 – 4:30 PM</td>
<td>Keynote Speaker – Barnett Rattner, USGS - “35 Year Historical Overview of CPRC-SETAC and the Field of Ecotoxicology”.</td>
</tr>
<tr>
<td>4:30 – 4:45 PM</td>
<td>Closing Remarks - Paula Henry, CPRC Past-President</td>
</tr>
<tr>
<td>4:45 – 5:00 PM</td>
<td>“Student Presentation Awards” – Darci Ferrer, CPRC President &amp; Ben Burruss, CPRC VP</td>
</tr>
</tbody>
</table>

* Student Presentation Award Competition
Poster Presentation (*Student Poster Award Competition)*:

1. **Jon Bartlett**, EAG Laboratories - Control Survival, Growth and Reproduction of the Marine Amphipod (Leptocheirus plumulosus) in a Flow-through Test System

2. **Shannon Edmonds**, UMD - Identifying and Evaluating the Major Ion Toxicity of Coal Combustion Residual Landfill Effluents

3. **Sarah Fischer**, UMD - Waste stabilization effects on biosolids-derived dissolved organic matter and complexation with emerging contaminants

4. **Anil Jajistar**, Towson University - Optimization of the lysozyme-like assay under low sample volume conditions for use in assessing immunotoxicity in small reptiles

5. **Victoria Lee**, Howard University - Effects of simulated coal ash leachate on the viability, behavior, and development of the seminole ramshorn snail (Planorbella duryi)

6. **Caroline Reed**, UNC-CH - A dysregulation of LPS-induced inflammatory response by mitotoxic Trimethyltin Hydroxide and Triethyltin Bromide

7. **Sue Thomas**, EAG Laboratories - Comparison of the use of natural vs. formulated sediment in sediment toxicity testing

8. **Caitlin Weible**, Towson University - Effects of Perfluorooctanesulfonic acid (PFOS) on Brown Anoles (Anolis Sagrei)
Quantification of water-air transfer rates for PCB’s and OCP’s in the Anacostia River using a passive sampling approach

Mandar Bokare (Department of Chemical, Biochemical and Environmental Engineering, University of Maryland Baltimore County, Baltimore, Maryland 21250, United States), Nathalie Lombard (Department of Chemical, Biochemical and Environmental Engineering, University of Maryland Baltimore County, Baltimore, Maryland 21250, United States), Samuel Magee (Department of Chemical, Biochemical and Environmental Engineering, University of Maryland Baltimore County, Baltimore, Maryland 21250, United States), Upal Ghosh (Department of Chemical, Biochemical and Environmental Engineering, University of Maryland Baltimore County, Baltimore, Maryland 21250, United States)

Polychlorinated biphenyls (PCB’s) and organochlorine pesticides (OCP’s) are major contaminants of concern in the Anacostia River, resulting in fish-consumption advisories in District of Columbia (DC). To quantify the sources and sinks for these pollutants, polyethylene (PE) passive samplers were deployed at several locations in DC to measure the freely dissolved and gas-phase concentrations of OCP’s and PCB’s. Our initial results show that the freely-dissolved PCB concentrations in the water column across the sites varied from 0.01 – 6.5 ng/L while measured OCP concentrations were in the range of 0.06 – 10.5 ng/L during the deployment period from March to July 2017. During the same period, gas phase PCB concentrations ranged from 222 – 1244 pg/m3, while gas-phase OCP concentrations were in the range of 89 – 119 pg/m3 across the sites. The water-air exchange flux for PCB’s and OCP’s over the Anacostia River was calculated to be +282 ng/m2/day and +273 ng/m2/day respectively, resulting in volatilization of about 355 g of PCB’s and 343 g of OCP’s per year from the Anacostia river. Ongoing work is estimating the total pollutant loads (including dissolved and particulate organic carbon associated loads) from Northeast and Northwest Branches of Anacostia and major tributaries, pollutant transfer rates between sediments and overlying water and the net pollutant outflow from the Anacostia into the Chesapeake Bay. Data analyses are ongoing for samples taken through a full one-year cycle to quantify seasonal differences that will allow a complete understanding of the annual pollution budgets for the Anacostia River.
Survival and biochemical health indicators of Elliptio complanata deployed in Anacostia River tributaries for monitoring of persistent organic contaminants

R.M. Harrison (University of Maryland College Park), A.E. Pinkney (US Fish and Wildlife), N. Lombard (University of Maryland Baltimore County), U. Ghosh (University of Maryland Baltimore County), L.T. Yonkos (University of Maryland College Park)

The Anacostia River is one of three regions-of-concern in the Chesapeake Bay Watershed. Persistent organic pollutants (POPS) such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and organochlorine (OC) pesticides are known to accumulate in sediment and biota within tidal/depositional portions of the Anacostia system, but on-going contaminant sources are poorly understood. The current project investigates relative contaminant contributions to the system by deploying freshwater mussels (Elliptio complanata) in non-tidal reaches of six Anacostia tributaries (plus an out-of-system reference site). The large adult mussels were meant to bioaccumulate POPs during 90-day cage deployments. Filter-feeding mussels can acquire dissolved and particle-bound contaminants during feeding, making them a useful tool for monitoring total POP loads transporting through the system. Additionally, E. complanata are indigenous to the Anacostia River, but few individuals remain due to stream blockage in the last century restricting migration of their host fish, the American eel. A second objective of the project was to determine the suitability of various tributaries of the Anacostia River for reintroduction of E. complanata now that eels have few restrictions to migration. Successful reintroduction would increase benthic community diversity and potentially improve water quality in the system. Year 1 results indicate very good survival of caged mussels during 90 and 150 day deployments with only one mortality out of 336 mussels deployed. Health indices (protein and carbohydrate) of 150-d deployed mussels also suggest conditions are amendable to mussel reintroduction. Tissues from 90-d deployed mussels are currently being analyzed for POPs. Results of analyses will be presented. Preliminary results of Yr-2 deployments will also be presented.
Activated carbon and biochar has grown in acceptance for in-situ treatment for polychlorinated biphenyl (PCB) and other persistent organic pollutants (POP) contaminated in sediments by reducing the freely dissolved pore water concentrations that drive aquatic food chain uptake. While decreasing availability to macro-organisms, carbonaceous materials have been demonstrated to enhance microbial and redox availability to sorbed contaminants. In addition to reducing aqueous PCB concentrations, activated carbon has also been evaluated as a possible delivery mechanism for both aerobic and anaerobic PCB degrading bacteria. Recent laboratory and pilot scale studies have been successful in demonstrating bioaugmented activated carbon as a viable treatment option for sediments contaminated with PCBs. The physiochemical properties of different pyrogenic carbon materials (coal AC, coconut hull AC, pinewood BC and graphite powder) have been demonstrated to have different effects of the dechlorination rate of PCB 61 to PCB 23 by the halorespiring bacteria Dehalobium chlorocoercia (DF-1) for in-situ treatment of PCBs by bioamended carbon. These results along with other developments offer a new hybrid approach for in-situ treatment of contaminated sediments and groundwater in the future.
Processing EPA’s ToxCast and Tox21 High Throughput Screening Data for Hazard Assessment

Patrick Raya (The George Washington University, Verisk 3e), Hans Plugge (Verisk 3e)

The objective of this study is to assess chemical hazards using data obtained from EPA’s ToxCast and Tox21 High Throughput Screening campaigns. The goal was to develop a process for listing chemicals and subsequently providing a metric to hazard endpoints. This was accomplished by screening an assortment of chemical data sets, from different use classifications, by biochemical enzyme assays specified by target organ and frequency of response. This basis acts as a listing function to flag toxic based on effects. Additionally, the data is interpreted by a scoring system (metric) focusing on Number of active hit and active hits versus total assays (Intensity Level), organ effect level, and data reliability determined in (human) in vitro assays. By scoring chemicals in this manner, a preliminary screening system is developed for flagging potentially toxic chemicals and mixtures, as well as providing a metric to their degree of toxicity.
Enterprise-wide Risk and Hazard Assessment

Hans Plugge (Verisk 3E, Bethesda, MD)

Hazard and risk assessments to date have primarily focused on single chemicals. Mixtures (aka products) assessment has mostly been limited to singling out the most hazardous component. Although chemicals in commerce are limited to somewhere less than 85,000 chemicals, the number of (retail) products is at least two - three orders of magnitude beyond that. Chemical Management especially at the retail level has focused on “forbidden” chemicals, i.e. focused only on inherent hazard. An example of hazard based decision-making would be the elimination of formaldehyde as a very low level preservative, way below the natural concentration of formaldehyde in an organic pear. Obviously the risks here are very low. By primarily focusing on hazard, manufacturers and retailers also lack information on the size of the risk incurred.

Sustainability can be thought of as optimizing risks: prioritize elimination of the big risks and work your way down to the smaller risks. Greening an enterprise thus proceeds in an organized fashion: rather than perception-based, “green” risk management can be (semi)quantitative.

Our approach proposes a (small) big data approach to combining hazard and inventory data into an enterprise wide database of environmental and health risk. We use two sources of data readily available at an enterprise level: GHS compatible SDS’s and inventory data.

First we abstract from the SDS the chemical composition of a product, resulting in a database of chemicals which can be scored using 3E Green Score, a hazard screening methodology for both environmental and health effects. Given that 3E Green Score scores mixtures, one can easily derive a hazard score for a mixture aka product. Using inventory data as a proxy/surrogate for exposure one can then calculate product based risk levels. Given all of the available information, one can also inventory single chemical quantities across all products and derive a risk score, again at the enterprise level.

The end result is two databases: product and chemical specific risk estimates at an enterprise level. Now one can apply hazard screening: all products containing “ungreen” chemicals or products with hazard scores below a threshold need reformulation – prioritization could occur via risk estimates.
Computational redesign of organophosphate flame retardants and pesticides for improved safety and efficacy

Traci Clymer (Department of Chemistry, George Washington University)

The current state of toxicity testing is inadequate for increasingly complex challenges, including growing number of complex endpoints, prediction of long-term effects from low-dose chronic exposure and the high cost of animal testing. There is a critical need to develop novel strategies and tools for the design of new commercial chemicals with reduced toxicity and acceptable functional efficacy. We assert that computational methodology employed to optimize drug candidates can be transformed to achieve this goal. Free energy perturbation (FEP) calculations are used to compute relative free energies of binding for molecular analogs to identify the most potent inhibitor in the process of lead optimization. By reversing this process, we can minimize binding to targets responsible for toxic effects by introducing subtle changes to the molecular scaffold.

As proof of concept, we focused on organophosphate (OP) flame retardants, an important class of chemicals with documented hazard concerns. The majority of OP flame retardants are triaryl phosphates. While structurally similar, OPs can lead to different toxic effects. For example, tricresyl phosphate (TCP) and triphenyl phosphate (TPP) differ only by the introduction of a methyl group; however, TCP is more neurotoxic than TPP, while TPP is considered an endocrine disruptor. Thus, both targets must be considered. To validate our computational approach, we relied on an established inhibitor of acetylcholinesterase (AChE), with reported IC50 values. Our combined Monte Carlo/FEP (MC/FEP) approach showed remarkable correlation between relative free energies of binding and relative IC50 values (R2 = 0.997).

Simple substituent scans on TPP have revealed that the placement of an electron-withdrawing group at the ortho position and/or an electron-donating group in the para position result in a marked decrease in binding affinity for AChE. The ideal groups to place in these positions will be selected to balance reduced affinity for AChE while minimizing the introduction of toxic metabolites. Functional efficacy for potential analogs will be determined as a function of phosphoric acid formation. Validation of our approach for OPs will allow for expansion to other relevant classes of chemicals with noted toxicity.
Establishing equilibration of chemical concentration in pore water and sediments prior to conducting sediment toxicity tests

Susan Thomas, Nanditha Billa, Henry Krueger (EAG Laboratories)

In sediment tests sufficient time is needed for a spiked chemical to equilibrate within the test system prior to exposing test organisms. Shorter equilibration times may be needed for chemicals that degrade more rapidly in sediment while longer periods are needed for those that are more persistent. For organic chemicals, it is recommended to age sediment at least one month before starting a test. For chemicals with a high log Kow (e.g., >6), a period of two months or longer may be necessary, and for metals, a shorter time frame of 1 to 2 weeks may be sufficient. OECD 218 recommends a period of 24 – 48 hours of equilibration prior to exposure; however, ASTM E1706-05 states that this short time period may not be long enough for sediments to equilibrate with the spiked chemicals. The EPA recommendation is that periodic monitoring of chemical concentration in the pore water during the sediment aging process should be used as a means to assess the equilibration of the spiked sediments. OCSPP 850.1000 suggests that flow-through systems may need to operate for a set period of time to obtain constant and representative concentrations. Another technique for determining equilibrium under testing conditions has been demonstrated by spiking the test chemicals into either natural or artificial sediments, mixing at least overnight to achieve homogeneity, adding the spiked sediment into the test beakers and then adding water in the test system. The pore water is analyzed after typically 2, 7, 10, 14, 21 and 28 days in the test system and the analytical results are examined for signs of equilibrium (when two intervals do not differ significantly) or significant loss (at or below the limit of quantitation). These results are used to choose an appropriate equilibration time for the definitive test. The results of this type of analysis for a variety of chemical types support the position that equilibration of a test chemical can be established within the test system instead of under storage conditions.
Potential for PFAS Cross-Contamination from Sampling Equipment and Associated Products

James Occhialini (Alpha Analytical), Elizabeth Denly (TRC), Phillip Bassignani (Alpha Analytical)

The potential presence of PFAS in common consumer products and in equipment typically used to collect environmental samples, coupled with the need for very low reporting limits heightens this concern. Can PFAS be transferred from common field and other commercial products during sampling? There are a lot of sampling recommendations in the literature but little actual data. In this study, the authors investigate what the potential for cross-contamination is from a number of commonly used products, with the emphasis on evaluating what the possible worst-case scenario for cross-contamination could be. PFTE, LDPE and HDPE tubing, pump bladders, and personal protective equipment are evaluated along with associated products such as aluminum foil and plastic storage bags.

In the experimental design of this study, the products themselves are not analyzed directly as in an assay. Rather, a series of experiments are performed utilizing a leaching model to evaluate the potential for cross-contamination and false positive environmental sampling results. All data is presented along with experimental observations and recommendations.
Challenges in Conducting Tier 1 Honey Bee Tests

Hank Krueger (EAG Laboratories)

Tier 1 Honey Bee studies required by EPA include older tests (acute oral, acute contact, foliage test) and newer tests (larval acute, larval chronic, and adult chronic). New tests were necessary to better understand how chemicals may affect bees in earlier stages of development (larvae and pupae) and how adult bees are affected by longer dietary exposure periods. The new tests have been challenging because handling and transfer of bees is much more demanding and requires new techniques and greater skill. For example, larval tests require the transfer of eggs from a frame out of a hive into well plates for the conduct of the test, while adult chronic tests require collecting newly emerged adults from a frame and transferring them to cages. Another challenge is confirmation of test concentrations in diets. This requires an understanding of the stability and homogeneity of the test material in the diet in order to maintain test concentrations in the test. The larval diet is difficult since it is a mixture of royal jelly, yeast, 2 sugars, and water that forms a viscous paste. The adult diet is sugar water and has limitations based on the solubility of the test material. As in most ecotox testing, it is the quality of the animals in cultures that determine the success of the test. Extensive hive management is necessary to produce strong queens, good egg laying and healthy hives. This is essential to achieve healthy test animals, good control survival and lower variability in tests.
Expanding the Number of Freshwater Invertebrates used in Acute Toxicity Tests for the Registration of Chemicals

D.S. Brougher, J.M. Griebel and, H.O. Krueger (EAG Laboratories Easton)

Abstract: There has been an increased interest in expanding the number of freshwater invertebrate species used for acute toxicity tests for the registration of chemicals. Tests for these species have followed guidelines based on the acute daphnia test that represents a water only exposure for 48 hours. Some tests have been extended to 96 hours. The battery of test we have conducted include the larval stage of freshwater insects (mayfly - Neocloeon triangulifer, caddisfly - Chimarra atterima, stonefly - Soyedina carolinensis), chironomids (C. riparius and C. dilutus), crayfish (Procambarus clarkii), freshwater amphipods (Gammarus pseudolimnaeus, Hyalella azteca), lumbriculus (Lumbriculus variegatus), and the daphnids (D. pulex and Ceriodaphnia dubia). Protocols for many of these species have often cited the daphnia guideline for acute testing. However, our experience in testing to date has determined that there are species specific ecological requirements and challenges (e.g. sediment/substrate requirements, cannibalism, feeding during testing) that are not being accounted for in the daphnia guideline. A need for species specific guidelines appears to be in order. Our recommendation is that the invertebrate guidelines should be revised in order to address the diversity of organisms being included in risk assessments.
Chemical mass balance source apportionment of trace metals in road dust

Matthew Fiala (Texas Southern University, Department of Environmental and Interdisciplinary Sciences) and Hyun-Min Hwang (Texas Southern University, Department of Environmental and Interdisciplinary Sciences)

Operation of motor vehicles is a major source of environmental contaminants, especially in urban areas. Trace metals are released through abrasive wear between brake pads and rotors; tires and pavement surfaces. Released trace metals become suspended in highway runoff and transported to receiving surface waters. To investigate the contribution of source materials to trace metals in road dust, pavement (asphalt and concrete) and road dust samples were collected from a highway (U.S. Route 59) in Houston, Texas. Upon arrival at the laboratory, road dusts were sieved to <63 µm to represent particles that become suspended by highway runoff. Bitumen and aggregates in asphalt pavement were separated using dichloromethane extraction. Total environmental-available trace metals were then quantified using ICP-MS. Concentrations for Pb, Cr, Ni, Cu and Zn were 95 ppm, 99 ppm, 160 ppm, 329 ppm, and 327 ppm, respectively, for concrete road dust and 40 ppm, 67 ppm, 120 ppm, 182 ppm, and 581 ppm, respectively, for asphalt road dust. Metal enrichment factors indicate that Cu and Zn are grossly contaminated in road dust. A chemical mass balance model reveals that primary sources of Cu and Zn are identified as brake and tire wear, respectively. Highway pavement material is identified as a significant source of Ni, Cr, and Pb in road dust, while abraded wheel weights are the primary source of Pb. The results of this study are important for developing environmental policies and practices to remediate water and sediment quality of urban streams.
Persistent organic pollutant monitoring in the Anacostia watershed using passive sampling and uptake in a freshwater mussel

N.Lombard (Department of Chemical, Biochemical, and Environmental Engineering, UMBC, Baltimore), M.Bokare (Department of Chemical, Biochemical, and Environmental Engineering, UMBC, Baltimore), V.Thodpanic (Department of Chemical, Biochemical, and Environmental Engineering, UMBC, Baltimore), SMagee (Department of Chemical, Biochemical, and Environmental Engineering, UMBC, Baltimore), R.Harrison (Department of Environmental Science and Technology, UMCP, College Park), L.Yonkos (Department of Environmental Science and Technology, UMCP, College Park), F.Pinkney (Chesapeake Bay Field office, USFW, Annapolis), U.Ghosh (Department of Chemical, Biochemical, and Environmental Engineering, UMBC, Baltimore)

Persistent organic pollutants such as polychlorinated biphenyls (PCBs), polynuclear aromatic hydrocarbons (PAHs) and organochlorine (OC) pesticides bioaccumulate in biota and pose a risk to human health through food consumption. Located within a major urban environment, the Anacostia River continues to receive inputs of legacy pollutants from its watershed, resulting in accumulation in fish and the issuance of fish consumption advisories. Ongoing Remedial Investigation/Feasibility Study of the tidal Anacostia is aimed at identifying the extent and magnitude of contamination, assessing risks, and evaluating cleanup alternatives. Model predictions are also required to determine the concentration reductions needed to achieve target tissue concentration to meet human health criteria. To address this aim, PCB, PAH, and OC pesticide concentrations in the water column and sediment porewater were monitored at several locations in the Anacostia River and major tributaries. A passive sampling approach was used and results were compared to quantify the dominant sources of pollution into the river. Caged mussels were concurrently deployed at the same tributary locations to measure in situ uptake in the tissue and determine if water concentrations are a good predictor for bioaccumulation at the lower trophic level of the food chain. Several organic pollutants levels in the Anacostia River water exceeded the EPA water quality criteria for the protection of human health. Our measurements indicate that some tributaries continue to serve as a source of the pollutants to the Anacostia River. Pollutant uptake was observed in mussel tissue over the three-month deployment period, in correlation with detected water concentrations. Partitioning-based bioconcentration models were tested and showed reasonable agreement within a factor of 2 to 10 between predicted and measured concentrations depending on the model tested. The overall results confirm that freely dissolved concentrations of persistent organic pollutants can be used to predict biouptake in benthic organisms. These results will help in determining the potential sources of contamination from tributaries into the River and developing better prediction of uptake in the aquatic food chain.
Liver tumor prevalence in bottom dwelling fish is widely used as an environmental indicator of biological exposure and response to carcinogens in sediments. Studies have shown that exposure to polynuclear aromatic hydrocarbons (PAHs) causes liver tumors in fish. Since 1992, the U.S. Fish and Wildlife Service has conducted tumor surveys of brown bullhead (Ameiurus nebulosus) from the Anacostia River (Washington, DC), tidal Potomac River watershed, and other Chesapeake Bay tributaries. We interpret results of 2014, 2015, and 2016 surveys and integrate these data into the 25-year database. The objectives are to: 1) compare tumor prevalence in bullheads from the Anacostia River and nearby areas across space and time; 2) analyze the 1992–2016 Tumor Data Base to identify reference locations and test age, length, weight, and sex as covariates; and 3) explore whether changes in bullhead exposure to contaminants may help explain the observed trends.

We report a large (>75%) statistically significant decrease in liver tumor probabilities for standardized 280 mm bullheads collected near the Anacostia CSX bridge between the 1996 and 2001 surveys (merged: female – 77.8%, male – 48.6%) and 2014–2016 surveys (female – 18.0%, male – 5.7%). Skin tumors, less clearly linked to contaminants, have also decreased dramatically in Anacostia bullheads. The 2014–2016 Anacostia CSX liver tumor prevalence is still significantly higher than that for the statistically-derived Chesapeake Bay Reference Group (CBRG; female: 9.4%, male: 2.7%). Liver and skin tumor prevalence, however, at Dyke Marsh, on the Potomac River ~5 km downriver from the Washington, DC border was low enough to be included in the CBRG. Sediment total and carcinogenic PAHs at two Anacostia bullhead survey locations have not changed substantially between 2000 and 2015. We interpret the large decrease in fish tissue polychlorinated biphenyls (PCBs) and DDT (both of which promote liver tumors in fish) as part of the explanation for the decreased tumor prevalence in Anacostia brown bullheads.
Leptocheirus plumulosus is an estuarine amphipod widely distributed in mesohaline waters along the eastern seaboard of the United States from Cape Cod, Massachusetts to northern Florida. Amphipods are representative of an important group of aquatic invertebrates, and are used in sediment toxicity tests based upon past history of use in the laboratory and the recommendations of the study guidelines (EPA 600/R-01/020 and EPA 600/R-94/025). In addition, the relatively short life span of L. plumulosus makes it ideally suited for evaluating chronic sub-lethal endpoints such as growth and reproduction.

The current USEPA guideline for the Leptocheirus plumulosus life cycle prescribes a static-renewal test design. The static-renewal design has resulted in minimal to no test organism survival and has difficulty meeting the acceptability requirements for a successful study. Consequently, EAG Laboratories has adapted a flow-through test design similar to that which is being used on chronic sediment toxicity tests with freshwater amphipods. By making a few changes to the study design, EAG Laboratories has become successful in running L. plumulosus life cycle toxicity tests. The volume of overlying water, frequency of overlying water exchanges and the passive exchange of overlying water over the sediment have improved the water quality in the test system. A change in feed type and an increase in feed rates have improved healthy growth in the organisms. The age of the organisms at the start of the study has helped improve the survival percentage. The changes made have enhanced the success rate for meeting the control validity criteria of the L. plumulosus life cycle test at EAG Laboratories.
Identifying and Evaluating the Major Ion Toxicity of Coal Combustion Residual Landfill Effluents

Shannon Edmonds (University of Maryland), Lance Yonkos (University of Maryland)

This project investigates the toxicological consequences of leachate from “dry” coal ash disposal on water quality and biota in receiving waters. The study includes laboratory-based toxicity testing of field-collected media. A quarterly series of whole effluent toxicity tests was completed on discharge samples from three Maryland disposal facilities. Results indicate varying levels of chronic toxicity at all three locations. Priority metals and major ions have been measured and monitored over time and concurrently with the bioassays. The wastewaters were found to be consistently high in total dissolved solids (TDS) and high conductivities were consistently observed, both indicative of elevated major ions. Traditional phase 1 toxicity identification evaluation (TIE) methods are not effective at addressing these issues. Therefore, the purpose of the present study is to evaluate the toxicity associated with major ion imbalances using mock effluents and a weight-of-evidence approach in order to determine and confirm the primary causative ions. Calcium, chloride, magnesium, sodium, potassium, and sulfate will be used to create a reconstituted mock effluent. A 7-day static renewal dilution series will be completed using Ceriodaphnia dubia and threshold inhibition concentration values will be compared between the reconstituted mock effluent and the wastewater. Historic effluent data will be compared with current effluent data across the three sites and seasonal patterns will be observed and noted. Recommendations for site improvements will be suggested.
Waste stabilization effects on biosolids-derived dissolved organic matter and complexation with emerging contaminants

Sarah Fischer (University of Maryland), Mark Ramirez (DC Water and Sewer Authority), and Alba Torrents (University of Maryland)

Final wastewater treatment solids, or “biosolids”, are a widely used agricultural soil amendment because they increase nutrient concentrations and water holding capacity of soils. Although the land-application of biosolids is a means of recycling, halogenated emerging contaminants (ECs) within biosolids are of concern for potential crop, groundwater, and ecological impacts. Environmental dissolved organic matter (DOM) has previously been shown to impact the general bioavailability and aqueous transport of hydrophobic contaminants and pesticides, by enhancing solubility from solid soil matrix. While it is well-established that DOM influences the fate and transport of organic contaminants in agricultural lands and water, limited work has characterized emerging contaminant (EC) interactions with DOM derived from biosolids. Furthermore, little is known about biosolids-derived DOM properties as a function of wastewater solids stabilization processes, including (i) anaerobically digested, (ii) aerobically digested, and (iii) limed biosolids.

Spectroscopic methods are currently being applied to characterize DOM from different solid-stabilization processes, and probe chemical interactions with halogenated ECs such as triclocarban (TCC) and degradation product and herbicide 2,4-dichlorophenol (2,4-DCP). Spectroscopic techniques such as UV-Visible, fluorescence, and Fourier-transform infrared (FTIR) spectroscopy have revealed differences in chemical moieties and operationally-defined functionalities, including fulvic-acid or humic-acid like, across different biosolids-derived DOM. Fluorescence spectroscopy was specifically employed in quenching experiments with DOM-contaminant solutions. Suppressed or quenched fluorescent DOM regions with an added contaminant indicate that TCC and 2,4-DCP have contrasting, pH dependent interactions with different biosolids-DOM. In limed biosolids-DOM, there appears to be more carbohydrate-like driven interaction, while in anaerobically digested material, more humic acid-like binding occurs. With the lack of a U.S. regulatory framework for organic contaminants in biosolids, this work supports the risk management science needed to assess the fate of emerging contaminants originating from land-application of biosolids.
Optimization of the lysozyme-like assay under low sample volume conditions for use in assessing immunotoxicity in small reptiles

Anil Jajista, Caitlin Weible, Andrew East, Christopher Salice (Towson University)

A key concern for many emerging contaminants, including per- and polyfluoroalkyl substances (PFAS), is whether sub-lethal effects occur as a result of environmental exposure. Understanding these sub-lethal responses is especially relevant for understudied taxa such as reptiles. Effects of toxicants on immune responses are especially relevant given the link between immune response and organismal health. Various PFASs have been shown in previous studies to suppress the innate immune response in rats, as determined with a lysozyme activity assay. The data from mammalian lysozyme activity assays, however, are based on relatively high blood volume samples and highly optimized sample preparation protocols. Smaller organisms such as Anolis lizards that have lower blood are more difficult to work with and lysozyme assays are likely prone to high margins for error. In order to quantify nonspecific immune response in Anolis lizards, we sought to further develop a lysozyme-like activity assay, which measures bacterial clearance by plasma extracted from blood samples. The assay involves UV-Vis detection, thus requiring calibration standards and sample to be of similar purity to decrease measurement uncertainty. Here, our ultimate goal was to optimize this assay for small lizards by adjusting the pH, the amount of bacteria added, the dilutions of sample, and the lysozyme concentration while using commercially available avian blood to improve current methods for low sample conditions. We have found that the most favorable conditions to improve signal-to-noise is to make all solutions at a pH of 6.2, adding bacteria in a 5:1 v:v ratio to the sample solution, and diluting the sample at 50% v:v and all while adjusting the concentration of calibration standards to better suit realistic lysozyme concentration in reptilian blood. Our modified conditions were better able to reliably analyze blood down to volumes of 60 μl, which will help to minimize sample dilution and pooling. Future studies will include introducing PFASs to the lysozymes to determine the effect on immune response and to evaluate immune response in lizards (Anolis sagrei) exposed to PFASs. We are hopeful that this assay will provide much needed immunotoxicity data for reptilian receptors.
Effects of simulated coal ash leachate on the viability, behavior, and development of the seminole ramshorn snail (Planorbella duryi)

Victoria Lee (Department of Biology, Howard University, Washington, DC 20059), Tyler E. Frankel (Department of Biology, Howard University, Washington, DC 20059)

Coal ash is one of the largest industrial waste products in the United States, and contains several toxic constituents such as selenium, mercury, and arsenic. These heavy metals have been detected in aqueous leachates from coal ash entering the environment via percolation through coal ash landfills and accidental spills. Exposure to coal ash leachate (CAL) has been associated with multiple detrimental effects in fish including increased mortality, decreased body condition, and decreased reproductive success. While coal ash spills have been shown to cause population decreases of invertebrate species inhabiting contaminated areas, few laboratory studies have been performed to ascertain lethal concentrations and determine the effects of exposure on specific reproductive, physiological, and morphological endpoints. This study investigated the effects of 0, 10, 30, 50, and 100 g/L CAL exposure on the viability, behavior, and development of the seminole ramshorn snail (Planorbella duryi), a hermaphroditic freshwater snail species. Adult snails were exposed to each treatment and mortality assessed over 72hrs. The ability of adult snails to detect and avoid coal ash contaminated water was assessed using a shuttle box assay. Newly hatched juveniles were continuously exposed to treatments for 120d, and effects on viability and shell width ascertained. After 72hrs, significant mortality was observed in the 100 g/L and 50 g/L treatments. Snails were able to detect and avoid water containing 30, 50, and 100 g/L CAL. Significant mortality rates were observed in the 100, 50, and 30 g/L treatments during the 120d juvenile exposure, and significant dose dependent decreases in shell width were observed in all treatments. While the assessment of lower (< 10 g/L) CAL concentrations is ongoing, our results suggest that further research examining the impacts on non-lethal endpoints is required to fully elucidate the impacts of CAL on P. duryi and other aquatic snail species.
A dysregulation of LPS-induced inflammatory response by mitotoxic Trimethyltin Hydroxide and Triethyltin Bromide

Caroline Reed (UNC-CH), Gabrielle Childers (NIEHS), Caroline Perry (NIEHS), and G. Jean Harry (UNC-CH, NIEHS)

When a eukaryotic body incurs trauma or infection, immune cells are prompted to orchestrate both defensive and curative inflammatory responses. Macrophage cells are implicated in inflammatory processes essential for elimination of pathogens, and required for homeostatic restoration. Mitochondrial dysfunction may represent a biological process and pathway by which environmental chemicals can modify the macrophage inflammatory response. Multiple organic tin compounds have been established as mitochondrial and neuronal toxicants by the NTP Tox21 program. Acute exposure to these chemicals can result in severe skin irritation, chronic neurological effects, and death. Although commonplace usage of these compounds has been prohibited in most developed nations, risks of occupational exposure is still relevant in many undeveloped industrial settings and antiquated infrastructures.

In order to evaluate how macrophage inflammatory response may be modulated by mitochondrial dysfunction, we employed two organotin compounds: Trimethyltin Hydroxide (TMT OH) and Triethyltin Bromide (TET Br). In this study, we explored how 6-hour pre-exposure to TMT OH or TET Br in murine RAW 264.7 macrophages may alter the basal LPS-driven inflammatory response. We saw TET Br pre-exposure elicit a mRNA blunting effect for both pro- (TNF, IL-1, and IL-10) and anti-inflammatory (IL-10) cytokines, where TMT OH did not. The inhibitory effect on cytokines of both nature may imply a holistic dysregulation of the inflammatory process within macrophages. In addition to these results, we saw a significant deviation in arginase II and TLR4 message levels in TET Br-dosed cells. Following this, a Mito Stress analysis revealed that TMT OH exposure did not significantly impact the oxygen consumption rate at any point during the experiment. However, in contrast, TET Br exhibited a significant difference from both the control and TMT OH groups during the period of basal respiration, ATP production, and maximum respiration. The reduction in maximal respiration also suggests a decreased spare respiratory capacity for TET Br-exposed cells. This data has provided a preliminary body of evidence indicating organotin-induced general inflammatory dysregulation and potential mitochondrial dysfunction.
Comparison of the use of natural vs. formulated sediment in sediment toxicity testing

*Sue Thomas, Nanditha Billa, and Henry Krueger (EAG Laboratories)*

Formulated sediments are mixtures of materials that mimic the physical components of natural sediments. Formulated sediments provide a basis by which any testing program can assess the acceptability of their procedures and facilities and provides a consistent measure of evaluating performance-based criteria necessary for test acceptability. The use of formulated sediment eliminates interferences caused by the presence of indigenous organisms, eliminates variation in sediment physico-chemical characteristics and provides a consistent method for evaluating the fate of chemicals in sediment. An acceptable formulated sediment should (1) support the survival, growth, or reproduction of a variety of benthic invertebrates, (2) provide consistent acceptable biological endpoints for a variety of species, and (3) be composed of materials that have consistent characteristics (consistent from batch to batch, contaminant concentrations below concentrations of concern and ingredients available to all individuals and facilities). Natural sediments should (1) come from a clean source, (2) be fully characterized, and (3) free of organisms that might compete with or consume the test organisms. An examination was performed on data generated by EAG Laboratories-Easton on the ability to meet control validity criteria for a variety of species used in sediment toxicity tests and specifically a comparison was made on which sediment type (natural vs. formulated) was used during the testing. Additionally, a comparison was made on the characterization of formulated and natural sediments used in the conduct of these studies. Similar results between sediments were observed for acute tests while some of the chronic tests performed dramatically did better with natural sediments.
Effects of Perfluorooctanesulfonic acid (PFOS) on Brown Anoles (Anolis Sagrei)

Caitlin Weible (Towson University), Anil Jajistar (Towson University), Andrew East (Towson University), and Christopher Salice (Towson University).

Per- and polyfluoroalkyl substances (PFASs) are persistent and abundant environmental contaminants, creating concern about their potential ecotoxicological effects on humans and wildlife. The goal of this research was to investigate the impacts of a specific PFAS known as perfluorooctanesulfonic acid (PFOS), on reptilian species. Intentional applications of PFOS in fire suppression training activities and their widespread use in many commercial products have resulted in their release into the environment where wildlife can be exposed. Unfortunately, there are no data available on the toxicity of PFOS to reptilian species precluding a robust assessment of risk to species in this taxa. To address this data gap, we used brown anoles (Anolis Sagrei) as the study species as they are abundant and invasive to many areas around the United States.

Lizards were dosed with PFOS using a pseudo-gavage method three times each week for a total of 35 days. A pseudo-gavage method was implemented to ensure accurate dose levels for each individual subject while also minimizing effects from excessive handling. A total of 40 lizards were used with 10 animals per dose which were 0, 0.02, 0.2, or 2 mg/kg per day. Dosing volumes were adjusted based on the weekly weight of each lizard. There were no lizard deaths during the 35 day study but there were apparent dose-related effects on change in lizard weight. All lizards were also necropsied and organs weighed. We found that as PFOS concentration increased, spleen and kidney masses increased. To our surprise, lizards in this study appeared to be similarly sensitive compared to avian receptors that had been exposed to PFOS in a reproductive study. Given the relatively short duration of the study (35 days), we hypothesize PFOS to exert greater toxicity with increasing exposure duration. The results of this study improve overall understanding of the effects on relevant wildlife of a widely distributed toxicant.
CPRC SETAC Sponsors

Sustaining Sponsors:

[Logos of sponsoring companies]
Associate Sponsors:

- ERM
- TRS
- ARCADIS
- BAT CONSERVATION INTERNATIONAL
- Science Traveler
- Environmental & Turf Services, Inc.

For CPRC SETAC sponsorship information please contact 2018 CPRC President – Darci Ferrer, (president.cprc.setac@gmail.com)