SOUTH CENTRAL CHAPTER-SETAC

2021

VIRTUAL ANNUAL MEETING

APRIL 16-17, 2021

South Central Region SETAC 2021 Virtual Annual Meeting

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Special thanks to our volunteers for your help in hosting our virtual meeting!
Megan, Kruuttika, Ashley, Kendall, Alexandra & Krisa

South Central Region SETAC 2021 Virtual Annual Meeting

Meeting Agenda

Thursday, April 15th (Zoom Meeting ID: 850 5641 8684; Password: SC2021)

6:00 – 7:30pm – Student Mixer/Networking

Friday, April 16th (Zoom Meeting ID: 821 2684 4301; Password: SC2021)

8:30 – 9:00am – Opening Comments from President of SC-SETAC

9:00 – 10:30am – Short-form Presentations

10:30 – 10:45am – Coffee Break

10:45 – 11:45am – Long-form Presentations *Chemistry & Exposure Assessment*

11:45 – 1:00pm – Lunch Break

11:50am Business Meeting (Zoom Meeting ID 996 1240 4323; Password: SC2021)

1:00 – 2:30pm – Long-form Presentations *Aquatic & Wildlife Toxicology*

2:30 – 2:45pm – Coffee Break

2:45 – 4:00pm – Long-form Presentations *Aquatic & Wildlife Toxicology*

4:00 – 5:00pm – Keynote Speaker Dr. Mike Honeycutt

Saturday, April 17th (Zoom Meeting ID: 954 9937 1252; Password: SC2021)

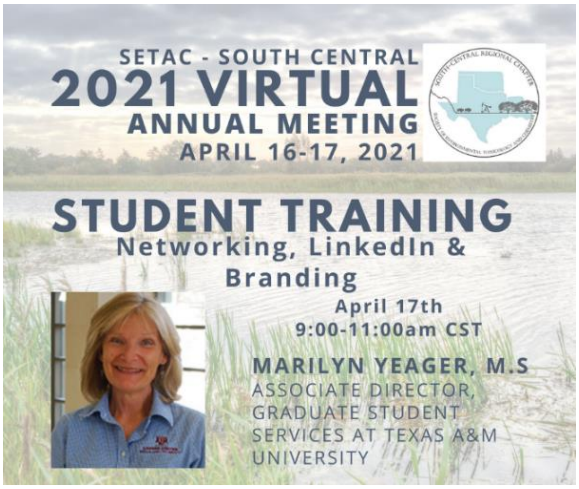
9:00 – 11:00am – Student Training Session

9:00 – 10:30am – Social Media Networking Bootcamp

10:30 – 10:45am – Break Out Rooms (Resume reviews)

10:45 – 11:00am – SETAC North America Info Session with Sarah Hughes

11:00 – 11:30am – Award Ceremony and Closing Comments



South Central Region SETAC 2021 Virtual Annual Meeting

Presentation Agenda & Abstracts

Short-form Presentation 9:00 – 10:30am

1. Kruuttika Satbhai - *Comparative Uptake and Depuration Kinetics of GenX and PFOA in Embryo-larval Zebrafish*
2. Megan Solan - *Perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) effects on phase I biotransformation enzymes in fish liver cells co-exposed to aryl hydrocarbon receptor (AHR) agonists*
3. Alexandra Cordava - *A Data Processing Workflow to Identify Structurally Related Compounds in Petroleum Substances Using Ion Mobility Spectrometry-Mass Spectrometry*
4. Ashley Ball - *In vitro cytotoxic effects of five parabens and their halogenated metabolites on fish and human hepatocytes*
5. Krisa Camargo - *Characterizing baseline legacy chemical contamination in urban estuaries for disaster-research through systematic evidence mapping: A case study*
6. Elizabeth DiBona - *Molecular assessment of environmental pollutant exposure during crucial innate immune system development windows in marine medaka (Oryzias melastigma)*
7. Cameron Emadi - *Effects of Co-Exposure to Hypoxia and Lead on Daphnia magna*
8. Sarah Nash - *Environmentally Relevant Concentrations of Anti-depressants and their Active Metabolites Attenuates Neurogenesis and Disrupts Larval Behavior in Zebrafish*
9. Farzana Hossain - *Acute Oral Toxicity of Non-fluorinated Fire Fighting Foams to Northern Bobwhite Quail (Colinus virginianus) Using the Up-and-Down Procedure*
10. Thomas Parkerton - *Elucidating Aquatic Toxicity-Cutoffs Using the Chemical Activity Paradigm*
11. Remi Labeille & Kelda Flores - *In silico computational chemistry to predict accessible and reactive areas for Benzo[a]pyrene metabolites in nucleosomes and DNA*
12. Brenna Butler - *Is crab exoskeleton a repository of the divalent heavy metal cadmium?*
13. Rachel Leads - *Co-exposure to crude oil and ultraviolet (UV) radiation induces cataract formation in fishes: a novel endpoint of photo-induced crude oil toxicity*
14. Rijith Jayarajan - *Role of miRNA regulation in Benzo[a]Pyrene- induced bone deformities in Medaka fish*

South Central Region SETAC 2021 Virtual Annual Meeting

Long-form Presentation 10:45 – 11:45am *Chemistry & Exposure Assessment*

1. Megan Woodyard - *A framework for assessing relative petrochemical vulnerability of marine fishes in the Gulf of Mexico*
2. Kamal Beagle - *Effect of Dual Exposure to Cigarette smoke and E-Cigarette Vapor on Respiratory Immunity*
3. Nicole Dennis - *Bioaccumulation in Northern Bobwhite Quail (Colinus virginianus) Liver Tissue and Egg Serum from Chronic Exposure to Perfluorohexanoic Acid (PFHxA) or a Mixture of PFHxA and Perfluorooctane Sulfonic Acid (PFOS)*

Long-form Presentation 1:00 – 2:30pm *Aquatic & Wildlife Toxicology*

4. Fabrizio Bonatesta - *Latent osmoregulatory defects in red drum (Sciaenops ocellatus) associated with early-life stage exposure to Deepwater Horizon crude oil*
5. Osaze Osayande - *Is crab exoskeleton a repository of the divalent heavy metal lead?*
6. Ashley Rea - *Melia Azedarach Fruit Toxicity*
7. Rachel Leads - *Influence of Cardiotoxicity on Visual Function in Developing Zebrafish (Danio rerio) Exposed to Deepwater Horizon Crude Oil*

Long-form Presentation 2:45 – 4:00pm *Aquatic & Wildlife Toxicology*

8. Amanda Csipak - *Toxicological Effects of Organic Contaminants in Hawksbill Sea Turtle Skin Cell Cultures*
9. Corey Green - *Effects of embryonic exposure to Aroclor 1254 on neurologic and cardiac endpoints in zebrafish (Danio rerio)*
10. Megan Mire - *Effects in vitro of 20-hydroxyecdysone and chrysene on hepatopancreatic expression of a CYP4 gene in the blue crab, Callinectes sapidus*
11. Marco Franco - *Reduced phase I biotransformation of polycyclic aromatic hydrocarbons (PAHs) in pollution-adapted Gulf killifish (Fundulus grandis)*

South Central Region SETAC 2021 Virtual Annual Meeting

Comparative Uptake and Depuration Kinetics of GenX and PFOA in Embryo-larval Zebrafish

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Perfluoroalkyl substances (PFAS) are receiving attention due to persistence in the environment and their adverse effects on animal and human health. After phasing out C8 PFAS, newer, shorter length replacement compounds have been introduced—such as perfluoro(2-methyl-3-oxahexanoic) acid (GenX). Little is known about the toxic effects of these replacement compounds; hence, it is important to understand to what extent these chemicals can enter aquatic organisms. We conducted a comparative analysis of Gen X and perfluorooctanoic acid (PFOA) in embryo-larval zebrafish (ZF). For the 24 hpf survival assay, the LC50 was 169.99 µM for GenX while the LC50 for PFOA was 81.84 µM. We assessed the uptake at 0-, 3-, 5- dpf, and depuration from 5–7 dpf. The larvae exposed to 20 µM of GenX or PFOA at 5 dpf showed internal concentration as high as 35.02 µM and 44.51 µM, respectively. However, after depuration GenX body burdens were reduced almost completely, up to 97.18% and 94.57% at 3.03 µM and 20 µM, respectively. PFOA was also depurated, though to a lesser extent, up to 35.58% and 49.51% for 3.03 µM and 20 µM, respectively. Estimated bioconcentration factors (BCF) for PFOA were higher than those for GenX, across concentrations and experimental duration. Our results demonstrate that GenX is less acutely toxic than PFOA to embryo-larval zebrafish.

Keywords: Chemistry and Exposure Assessment, Aquatic Toxicology

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Perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) effects on phase I biotransformation enzymes in fish liver cells co-exposed to aryl hydrocarbon receptor (AHR) agonists

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The presence of perfluorinated substances in the environment, especially in aquatic ecosystems, continues to be of concern for human and environmental health. Previous studies have suggested that several of these compounds do not undergo biotransformation due to their chemical stability, yet PFOS- and PFOA-exposed organisms have presented abnormal activity of important biotransformation pathways. Given the fundamental role of biotransformation in biological organisms and the significant distribution of perfluorinated substances in aquatic environments, the present study was designed to investigate the influence of PFOA and PFOS on phase I biotransformation enzymes in fish using the rainbow trout liver RTL-W1 cell line and the minnow liver cell line PLHC-1. Cells were exposed and co-exposed to environmentally relevant concentrations of PFOA, PFOS, and Benzo[a]pyrene (BaP). Cells were evaluated for cytotoxicity and activity of CYP1A, via EROD activity bioassays, following a 48 h exposure. Preliminary data suggests that PFOS but not PFOA decrease CYP1A activity even in presence of agonists (BaP) in liver cells. These observations have significant implications for organisms that may be exposed to other environmental pollutants for which biotransformation is necessary, especially in detoxification mechanisms. The inability of biotransformation pathways to function as needed could significantly increase adverse outcomes, compromising the stability of fish populations inhabiting PFOA- and PFOS-polluted environments.

Keywords: Chemistry and Exposure Assessment, Aquatic Toxicology, Environmental Risk Assessment

South Central Region SETAC 2021 Virtual Annual Meeting

A Data Processing Workflow to Identify Structurally Related Compounds in Petroleum Substances Using Ion Mobility Spectrometry-Mass Spectrometry

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Ion mobility spectrometry coupled with mass spectrometry (IMS-MS) is a high-resolution post-ionization separation technique that can be used for rapid analysis of complex samples. IMS-MS offers untargeted analysis, including ion-specific conformational data derived as collisional cross-section (CCS) values. Here we combine CCS and Kendrick Mass Defect (KMD) analyses based on CH2 and H functional units to enable chemical compositional analyses of petroleum substances. First, polycyclic aromatic compound (PACs) standards were analyzed by IMS-MS to demonstrate how CCS assists identification of isomeric species in homologous series. Next, we used a case study of a gasoline standard previously characterized for paraffin, isoparaffin, aromatic, naphthene, and olefinic (PIANO) compounds to demonstrate the application of various KMD analyses and CCS filtering parameters. Finally, we propose a workflow that enables confident molecular formula assignment to >96% of the IMS-MS-derived features in the gasoline standard. Collectively, this work demonstrates how rapid untargeted IMS-MS analysis and the proposed data processing workflow can be used to provide confident compositional characterization of complex petroleum samples.

Keywords: Chemistry and Exposure Assessment, Environmental Risk Assessment

South Central Region SETAC 2021 Virtual Annual Meeting

In vitro cytotoxic effects of five parabens and their halogenated metabolites on fish and human hepatocytes

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Parabens, used as artificial preservatives in cosmetic and body care products, were of serious concern in human toxicology in the early 2000s due to their potential for estrogenic activity. That concern was determined to be unfounded using predominantly human or mammalian models, and paraben use has been continued with few signs of slowing. There has been a renewed interest in parabens recently due to halogenated parabens' discovery as a byproduct of common wastewater treatments. Several brominated parabens have been found in freshwater, suggesting more research into these compounds' safety needs to be carried out. Previous work in our lab demonstrated that G1B (catfish gill) cells had the potential for more sensitive cytotoxic responses to parent parabens (methyl-, ethyl-, propyl-, butyl- and benzylparaben) compared to other cell lines. In this study, an LC50 at 96 h of exposure was calculated for those five parent parabens; three halogenated parabens and one primary metabolite was calculated in these cells as well as in rainbow trout hepatocytes (RTL cells), rainbow trout gill cells (RTgill-W1 cells), human hepatocytes (HepaRG cells) and human intestinal cells (CaCo2 cells).

Keywords: Environmental and Wildlife Toxicology

South Central Region SETAC 2021 Virtual Annual Meeting

Characterizing baseline legacy chemical contamination in urban estuaries for disaster-research through systematic evidence mapping: A case study

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Natural disasters such as floods and hurricanes impact urbanized estuarine environments. Some impacts pose potential environmental and public health risks because of legacy or emerging chemical contamination. However, characterizing the baseline spatial and temporal distribution of environmental chemical contamination before disasters remains a challenge. To address this gap, we propose using systematic evidence mapping (SEM) in order to comprehensively integrate available data from diverse sources. We demonstrate this approach is useful for tracking and clarifying legacy chemical contamination reporting in an urban estuary system. We conducted a systematic search of peer-reviewed articles, government monitoring data, and grey literature. Inclusion/exclusion criteria are used as defined by a Condition, Context, Population (CoCoPop) statement for literature from 1990-2019. Most of the peer-reviewed articles reported dioxins/furans or mercury within the Houston Ship Channel (HSC); there was limited reporting of other organics and metals. In contrast, monitoring data from two agencies included 89-280 individual chemicals on a near-annual basis. Regionally, peer-reviewed articles tended to record metals in Lower Galveston Bay (GB) but organics in the HSC, while the agency databases spanned a wider spatial range in GB/HSC. This SEM has shown that chemical data from peer-reviewed and grey literature articles are sparse and inconsistent. Even with inclusion of government monitoring data, full spatial and temporal distributions of baseline levels of legacy chemicals are difficult to determine. There is thus a need to expand the chemical, spatial, and temporal coverage of sampling and environmental data reporting in GB/HSC.

Keywords: Chemistry and Exposure Assessment

Molecular assessment of environmental pollutant exposure during crucial innate immune system development windows in marine medaka (Oryzias melastigma)

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The innate immune system is critical for pathogen defense, organism homeostasis and tumor suppression. Despite being a prominent target for environmental pollutants, quantification of immune compromise is challenging due to the complex interactions of multiple pathways. In-line with the 'Developmental Origins of Health and Disease' (DOHaD) hypothesis, there is increasing evidence that exposure to toxicants during critical phases of immune system development is associated with later-life immune disease susceptibility. Marine medaka (Oryzias melastigma) have previously been highlighted as a model for developmental immunotoxicity. However, knowledge of onset and functionality of innate immune parameters such as, immune initiators, mediators, and effectors is still missing. To assess occurrence and functionality of selected innate immune genes, gene expression was measured during embryonic development (5-11 days post fertilization (dpf)) with/without pathogen challenge. Results indicated a potential critical window of innate immune development between 7 and 11 dpf. Gene expression was significantly reduced for immune initiators (C1q, TLR5-soluble) and immune mediators (MYD88, M-CSF) at 11 dpf. In contrast, the immune effector, lysozyme, increased in expression significantly from 5 to 11 dpf. Pathogen challenge did not affect the measured immune gene expression. The identification of molecular markers for developing innate immune response provides the baseline to identify susceptible developmental pathways and stages. Subsequent exposure experiments targeting these critical innate immune system development stages combined with later-life stage immunocompetence assessment will provide a high-throughput toolbox to assess immunotoxicity of common environmental pollutants.

Keywords: Aquatic Toxicology, Environmental and Wildlife Toxicology

Effects of Co-Exposure to Hypoxia and Lead on Daphnia magna

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Generating an understanding of how stressors in the environment interact is important for assessing environmental toxicity. Hypoxia is a stressor that impacts both fresh and saltwater ecosystems and is increasing in severity and prevalence due to anthropogenic factors. Lead (Pb), a non-essential metal and pervasive pollutant, represents another source of stress in aquatic environments; yet, little is known regarding the potential interactive effects of co-exposure to hypoxia and Pb in aquatic animals. Our study focuses on the acute effects of co-exposure to hypoxia and Pb on Daphnia magna. In response to hypoxia, D. magna have been shown to elevate ventilation and heart rates, increase hemoglobin (Hb) concentration and upregulate certain isoforms of hemoglobin. Exposure to Pb has been shown to decrease Hb production. The significance of Hb in convective oxygen transport within D. magna presumably changes with development, becoming more important with growth beyond the critical size limit for purely diffusive oxygen transport (< 1 mm in normoxia). Thus, sensitivity to Pb might be related to oxygen transport effects that likely vary with age-related size differences and oxygen availability. To investigate, acute (48-h) mortality bioassays were performed for different life stages of D. magna (neonates and adults) using Pb exposures in normoxia and hypoxia. To gain insight of the potential role of Pb-induced impairment on convective oxygen transport in contributing to the observed toxicity, Hb concentrations and oxygen consumption rates were measured. This data should aid in predicting the toxicity of Pb to D. magna in conditions of environmental hypoxia.

Keywords: Chemistry and Exposure Assessment, Aquatic Toxicology

South Central Region SETAC 2021 Virtual Annual Meeting

Environmentally Relevant Concentrations of Anti-depressants and their Active Metabolites Attenuates Neurogenesis and Disrupts Larval Behavior in Zebrafish

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Venlafaxine and Citalopram are widely prescribed antidepressants that are selective serotonin and norepinephrine reuptake inhibitors (SSNRI's). Both parent compounds and their active metabolites (o-desmethylvenlafaxine and n-desmethylcitalopram) are found in aquatic environments around the United States with concentrations ranging from 1 ng/L to 500 ng/L. Although these compounds occur in the environment at lower levels, they still may be more bioactive at those concentrations. There are several studies that have shown the impacts of the parent compounds on juvenile fish behavior and morphology, but there is little information on the impacts of their active metabolites at environmentally relevant concentrations. In this study, we tested the hypothesis that o-desmethylvenlafaxine and n-desmethylcitalopram disrupt molecular mechanisms responsible for serotonin reuptake and developmental programming using zebrafish (Danio rerio). We analyzed zebrafish behavior by evaluating white light startle response, as well as hatching success at 54 hpf and morphology post-exposure to Venlafaxine and Citalopram as well as their metabolites, o-desmethylvenlafaxine and n-desmethycitalopram, at various concentrations ranging from 0.1nM to 10 µM. The goal of this study was to assess if the serotonin reuptake signaling pathway was impacted upon exposure to these active metabolites. To the best of our knowledge, this study reports the first findings on the impacts of o-desmethyvenlafaxine and n-desmethycitalopram in zebrafish.

Keywords: Chemistry and Exposure Assessment, Aquatic Toxicology

South Central Region SETAC 2021 Virtual Annual Meeting

Acute Oral Toxicity of Non-fluorinated Fire Fighting Foams to Northern Bobwhite Quail (Colinus virginianus) Using the Up-and-Down Procedure

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Per-and poly-fluoroalkyl substances (PFAS) are the active ingredients in fire fighting foams. Long-chain PFAS are extremely persistent in the environment, and the regulatory agencies are concerned about their potential adverse environment and health impacts. Recently, non-fluorinated chemical constituents have been proposed for use in fire fighting foams in an effort to reduce the potential negative impacts of PFAS on terrestrial and aquatic flora and fauna. However, it is important to also determine the potential ecotoxicity of these non-fluorinated foam products. We conducted an acute oral toxicity test using six different fluorine-free foams (BIOEX ECOPOL, FOMTEC ENVIRO USP, NRL 502W Foam, SOLBERG, National Foam AVIO F3 and NFD) and one short-chain fluorinated foam (BUCKEYE) to Northern Bobwhite Quail (Colinus virginianus). Groups of 5 birds were initially pseudo-gavaged with a volume of each product corresponding to a “limit” (the highest concentration expected to occur environmentally). Only one bird (1 of 35) died during the limit test, indicating that all 7 products have an acute LD50 in adult quail at or above the limit (~ 1500 mg/kg body weight). In preparation for a chronic study with exposure via drinking water, we also completed a water avoidance trial.

Keywords: Chemistry and Exposure Assessment

South Central Region SETAC 2021 Virtual Annual Meeting

Elucidating Aquatic Toxicity-Cutoffs Using the Chemical Activity Paradigm

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Chemical activity (CA) has been proposed as a novel exposure metric for expressing aquatic toxicity data and facilitating single substance as well as mixture hazard/risk assessments since CA serves as a proxy of toxic units. CA can be estimated by dividing the freely dissolved concentration of a substance in water by the corresponding sub-cooled solubility limit of the chemical. Based on this paradigm, baseline toxicity is reported to occur at CA > 0.01. This paradigm provides a mechanistic basis to explain the observation that some solids do not cause toxicity at the solubility limit since the corresponding maximum CA is below this threshold. However, this implies that all hydrophobic liquids will exhibit toxic effects if tested at the solubility limit (i.e. CA=1). Reliable toxicity data to critically test this hypothesis have been lacking given the difficulty of delivering, maintaining and confirming test exposures at CA=1. To address this challenge, vapor and passive dosing methods have been applied to investigate the toxicity of various classes and chain lengths of hydrophobic liquids using algae growth and daphnid chronic endpoints in single exposure limit tests at the solubility limit. Results showed empirical toxicity-cutoffs are observed across substance classes as chain length increased despite CA exposures of unity. These findings illustrate the utility of coupling effective dosing strategies with limit studies for practically defining toxicity cutoffs. Shortcomings of applying this experimental design and CA concepts in toxicity assessment of hydrophobic substances will be discussed

Keywords: Aquatic Toxicology

South Central Region SETAC 2021 Virtual Annual Meeting

In silico computational chemistry to predict accessible and reactive areas for Benzo[a]pyrene metabolites in nucleosomes and DNA

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Benzo[a]pyrene (BaP) and its metabolite benzo(a)pyrene-7,8-dihydrodiol 9,10-epoxide (BaP-DE) have been observed to induce transgenerational bone impairment in fish through epigenetic profile modifications in the germ cells. It is hypothesized, that both DNA methylation and histone tail modifications are contributing factors to generate the observed transgenerational phenotype. To determine DNA loci and nucleosome molecule structures susceptible for BaP-DE binding, and subsequent changes of DNA methylation and histone tail modifications, a holistic in silico computational chemistry pipeline has been developed:

a) To provide insight how binding affinity to DNA hotspots will impact the DNA structure and gene expression, DNA methylation at CpG islands is analyzed for BaP-DE binding affinities. Model DNA sequences are created with Avogadro, using CG and AT base pairs alone and in combination to characterize the interaction and affinity with BaP-DE using AutoDock Vina and Abalone. Top-ranked sequences for binding will be analyzed for conformational changes impacting DNA re-/de-methylation, and thus, subsequently modifying gene expression.

b) To assess possible BaP-DE binding sites at the histone tails H3 and H4 and interference with post-translational modification (PTM) the software AutoDock Vina was employed. The BaP-DE/histone tail complexes are then examined for conformational and functional changes which may affect gene expression, using the molecular dynamics package (hybrid Monte Carlo approach) available in Abalone software.

Together, these in silico computational chemistry analyses will allow to identify priority sites for study in germ cells and osteoblasts in the biological Medaka fish model and enhance the understanding of epigenetic mechanisms involved in transgenerational inheritance.

Keywords: Chemistry and Exposure Assessment

South Central Region SETAC 2021 Virtual Annual Meeting

Is crab exoskeleton a repository of the divalent heavy metal cadmium?

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The post-ecdysial mineralization in crustaceans involves deposition of carbonate salts, such as calcium carbonate, to the organic matrix in the new exoskeleton. Cadmium is a toxic, non-essential metal frequently found in tissues of aquatic crustaceans. The purpose of this project was to determine whether cadmium is incorporated into the new shell during the post-ecdysial mineralization using the blue crab, Callinectes sapidus as the model crustacean. It was hypothesized that the injected cadmium would be deposited into the shell by calcium transporters in the epidermis during the mineralization process because of the resemblance between cadmium and calcium ions. To test this, soft shell blue crabs were injected with cadmium chloride, and cadmium content in the exoskeleton was quantified using ICP-OES. As carbonic anhydrase is an important enzyme in the mineralization process, its activity levels were also analyzed for both groups, although no change was noticed. The results suggested that during the mineralization process, blue crabs can incorporate cadmium into their exoskeleton as they would calcium. Cadmium was present in the exoskeleton of the control group, the exuviae, and the treated group in a significantly higher concentration. The presence of cadmium in control crabs and exuviae and the amplification of cadmium content in treated crabs suggests that crab shells can be used as biomarkers for cadmium pollution. A significantly smaller exoskeleton weight in the treated crabs was observed, suggesting that Cd treatment has a detrimental effect on the formation of the organic matrix of the exoskeleton.

Keywords: Aquatic Toxicology, Environmental and Wildlife Toxicology

South Central Region SETAC 2021 Virtual Annual Meeting

Co-exposure to crude oil and ultraviolet (UV) radiation induces cataract formation in fishes: a novel endpoint of photo-induced crude oil toxicity

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

Keywords: Aquatic Toxicology, Environmental and Wildlife

Role of miRNA regulation in Benzo[a]Pyrene- induced bone deformities in Medaka fish

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Benzo[a]Pyrene (BaP) is an ubiquitously detected polycyclic aromatic hydrocarbon in the environment. It has been demonstrated that BaP can cause bone deformities in a transgenerational manner in Medaka fish (Oryzias latipes). Ancestral BaP exposure has been shown to alter the expression of microRNA (miRNA) - target gene pairs during bone development. In this study, a microinjection-based approach was used to screen the key microRNAs (miR214, miR199a, miR210) involved in BaP-induced skeletal defects. Custom made oligonucleotides for agonists and antagonists of each miRNA were injected at 3 days post fertilization (dpf). The calcified bone area and calcification intensity were assessed every third day until 30 dpf in the control, miR-agonist, and -antagonist groups. A reduced bone calcification was observed upon miR199a agonist injection at 27/30 dpf, indicative of its role during late bone development. MiR214 agonist injection resulted in an increased calcification in the posterior vertebrate region at 12 dpf indicating its role during early bone development. Injection of miR210 agonist or antagonist did not reveal any changes in bone calcification, but a high mortality rate was observed in miR210-agonist/-antagonist injected fish after 12 dpf. The results indicate that increased concentrations of miR199a and miR214 may contribute to transgenerational BaP-induced bone toxicity. The data confirm a functional similarity of miR-199a and miR-210 between medaka fish and mammals. The present study highlights two different developmental timepoints potentially crucial for normal bone development in fish and vertebrates. Follow-up experiments will address molecular and cellular changes induced by deregulation of these miRs.

Keywords: Aquatic Toxicology

South Central Region SETAC 2021 Virtual Annual Meeting

A framework for assessing relative petrochemical vulnerability of marine fishes in the Gulf of Mexico

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In the Gulf of Mexico, oil spills are a significant threat to biodiversity. Crude oil is an extremely harmful toxicant to fish, with many short-term and long-term effects. Attempts to evaluate impacts post-spill are often stymied by the lack of an efficient mechanism to quantify countless species' relative vulnerabilities to petrochemicals for community and population impact assessments, effective allocation of resources, etc. Comprehensive toxicity testing is largely unavailable for most Gulf fishes. One solution is a Vulnerability Index of selected, scored trait-based indicators to model species' relative vulnerabilities to stressors like an oil spill. A multi-taxonomic petrochemical vulnerability framework for marine vertebrates and select groups of invertebrates was recently created by 28 international scientists representing a breadth of expertise in species' sensitivities to petrochemicals, marine ecology, behavior, taxonomy, pharmacokinetic modeling, molecular mechanisms, and petrochemical environmental chemistry. This framework was applied to the complete list of 1,670 bony and cartilaginous fishes present in the Gulf of Mexico, with six traits predicting likelihood of exposure, species sensitivity, and population resilience to best determine Gulf fishes' relative vulnerability to oil spills. The most vulnerable species were identified, all fishes' vulnerability classified, and accompanying patterns elucidated. This vulnerability framework of Gulf of Mexico fish species will facilitate rapid and effective action to prioritize areas for real-time responses to prevent oil contamination in areas with high numbers of vulnerable species, and after spills, to identify species most likely impacted by oil spills.

Keywords: Chemistry and Exposure Assessment, Environmental Risk Assessment

South Central Region SETAC 2021 Virtual Annual Meeting

Kamal Beagle - Effect of Dual Exposure to Cigarette smoke and E-Cigarette Vapor on Respiratory Immunity

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Background: Vaping has eclipsed cigarette smoking in the last decade, with the teen vaping epidemic affecting high school and middle school students being described as ‘addiction crisis” by the FDA. Although e-cigarettes were originally marketed as smoking cessation devices, dual users who simultaneously smoke cigarettes and vape have also been steadily increasing. While the e-cigarette vapor may contain fewer toxic chemicals than regular cigarette smoke, multiple studies have shown EV exposure to be causally linked with adverse health effects in humans, an issue that is further emphasized by the recent outbreak of vaping-associated severe respiratory failure resulting in ~3000 hospitalizations and 68 confirmed deaths. The primary objective of this study is to delineate effects of dual exposure on respiratory immune defenses against bacterial infection.

Method: We exposed 6-8 weeks old C57BL/6 mice to cigarette smoke (CS), e-cigarette vapor (EV) or to both cigarette smoke and e-cigarette vapor (dual) for 4 weeks. After last exposure, we inoculated mice with Streptococcus pneumoniae strain TIGR4 via intranasal route. At 24h post-infection we enumerated bacterial CFUs and examined cytokine expression (qPCR and ELISA) in mouse nasal septa, broncho-alveolar lavage fluid (BALF), and lungs.

Result: At 24h post-infection, the bacterial burden in mouse tissues in from different exposure cohorts was similar. Although, expression of pro-inflammatory cytokine IL-6 was significantly suppressed in dual exposed mice infected with S. pneumoniae compared with their counterparts exposed to CS or EV alone.

Conclusion: In summary, dual exposure to CS and EV suppresses mouse pulmonary pro-inflammatory immune response. We plan to examine the effects of long-term (8 and 16 week) dual exposure on pulmonary immune defenses against respiratory pathogens.

Keywords: Chemistry and Exposure Assessment, Environmental Risk Assessment

South Central Region SETAC 2021 Virtual Annual Meeting

Bioaccumulation in Northern Bobwhite Quail (Colinus virginianus) Liver Tissue and Egg Serum from Chronic Exposure to Perfluorohexanoic Acid (PFHxA) or a Mixture of PFHxA and Perfluorooctane Sulfonic Acid (PFOS)

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Characterizing the chronic toxicity of per- and polyfluoroalkyl substances (PFAS) is currently necessary because there are epidemiological and toxicological studies that presently report adverse health effects due to PFAS exposure, and PFAS are globally present in nearly every environmental compartment tested over the past two decades. The sparse PFAS toxicology data still presently available particularly for terrestrial ecological receptors continues to hinder the development of chronic PFAS toxicological reference values (TRVs) that risk assessors use to perform ecotoxicological risk assessments (ERAs) of PFAS-impacted environments. Therefore, in an ongoing effort to characterize the chronic oral toxicity of select individual PFAS and binary PFAS mixtures to avian ecological receptors, the present study extracted, quantified, and reported the PFAS residues from liver tissue and eggs from avian specimens previously exposed to perfluorohexanoic acid (PFHxA) and a mixture of PFHxA and perfluorooctane sulfonic acid (PFOS). From the previously reported dose-dependent no-observable-adverse-effect-level (NOAEL) and/or lowest-observable-adverse-effect-level (LOAEL) chronic oral exposure thresholds to PFHxA and PFOS:PFHxA we presently estimated and currently report a liver tissue and whole egg homogenate chronic toxicity value (CTV) corresponding to the NOAEL and/or LOAEL thresholds for avian reproduction, growth, and offspring survival. We further compared these values across previous similar exposure studies for a more detailed understanding of the modes of toxicological action of PFAS to avian receptors. The species-and tissue-specific NOAEL and/or LOAEL CTVs provided herein (representative of possible population-level adverse health effects) are immediately useful for TRV development and assessing reproductive health risk to wild avian receptors at PFAS-impacted sites.

Keywords: Chemistry and Exposure Assessment, Environmental Risk Assessment

Fabrizio Bonatesta - Latent osmoregulatory defects in red drum (Sciaenops ocellatus) associated with early-life stage exposure to Deepwater Horizon crude oil

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Crude oil is known to induce developmental defects in teleost fish exposed during early-life stages (ELSs). Those associated with the heart are believed to elicit a characteristic suite of downstream effects (e.g., pericardial and yolk sac edema) that likely impair the proper development of other organs. As evidence, zebrafish (Danio rerio) larvae acutely exposed to Deepwater Horizon (DHW) crude oil showed transcriptional changes in key genes involved in early kidney (pronephros) development and function that were coupled with pronephric morphological defects. Given the osmoregulatory importance of the kidney, it is unknown whether ELS effects arising from short-term crude exposures result in long-term osmoregulatory defects, particularly within estuarine fishes likely exposed to DWH oil following the spill. To address this knowledge gap, a 24h exposure to red drum (Sciaenops ocellatus) larvae was performed using high-energy water-accommodated fractions (HEWAFs) of DHW slick oil. Red drum were subsequently transferred to clean seawater for two weeks and a 96h acute salinity transfer test was performed by exposing the fish to waters with varying salinities. Latent effects of ELS crude oil exposure on osmoregulation were assessed by quantifying survival during the acute salinity transfer test. Additionally, transcriptional changes in genes with various structural, functional and signaling roles specific to different regions of the pronephros were assessed by QPCR. Results demonstrated that ELS crude oil exposure impaired the ability of red drum larvae to properly osmoregulate in hypoosmotic waters and that transcriptional changes in some target genes were evident following several days of HEWAF exposure.

Keywords: Aquatic Toxicology, Environmental and Wildlife Toxicology

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Is crab exoskeleton a repository of the divalent heavy metal lead?

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Lead is a toxic, non-essential metal frequently found in tissues of aquatic crustaceans. It gets into the aquatic ecosystem through a wide range of natural and anthropogenic sources. The post-ecdysial mineralization in crustaceans involves the deposition of carbonate salts, such as calcium carbonate, to the organic matrix. Due to the resemblance between lead and calcium ions, this study was carried out to investigate whether lead is incorporated into the new shell during post-ecdysial mineralization using blue crab, Callinectes sapidus, as the model crustacean. It was hypothesized that injected lead would be deposited in the shell via calcium transporters in the epidermis during the mineralization process. Post-ecdysial blue crabs were injected with 5 µg Pb/g wet weight in lead acetete solution and lead and calcium content were analyzed in the exoskeleton, blood, hepatopancreas, gills and muscles. Results showed a non-significant increase (P_>_0.05) in exoskeletal lead content in lead treated crabs compared to control. Interestingly, lead was found present in the exoskeleton of control crabs indicating that these crabs were previously exposed to lead in their previous habitat. There was a significant decrease in calcium content (P_<_0.05) in lead treated crabs suggesting that lead hinders the deposition of calcium to crab exoskeleton thereby obstructing calcification. There was a significant increase of lead found in the gills, hepatopancreas, muscle and blood in lead treated crabs. The rank of the Pb level amongst three soft tissues in a decreasing order is: hepatopancreas > gill > muscle > blood. This study is the first to present evidence that lead can obstruct calcium deposition in post-ecdysial crustaceans.

Keywords: Aquatic Toxicology, Environmental and Wildlife Toxicology

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Melia Azedarach Fruit Toxicity

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Melia azedarach, commonly known as Chinaberry, is a non-native, exotic tree declared invasive in the United States and globally. This deciduous tree threatens ecosystem stability due to its rapid growth rate, resilience to management, and toxicity to various native species. Recent literature focuses on the antifeedant, insecticidal, and antimicrobial tendencies of Chinaberry extracts rather than whole plant matter. Our research analyzed the toxicity of local Melia azedarach fruit to western freckled crayfish (Faxonius occidentalis). The effects of exposure to varying berry concentrations was evaluated by mortality rate and responsiveness. Laboratory tests displayed high mortality when exposed to as few as three berries in a 10 L tank. Toxicity to intermediate predators like crayfish could have drastic effects on watersheds invaded by Chinaberry.

Keywords: Aquatic Toxicology, Environmental and Wildlife Toxicology

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Influence of Cardiotoxicity on Visual Function in Developing Zebrafish (Danio rerio) Exposed to Deepwater Horizon Crude Oil

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Polycyclic aromatic hydrocarbons (PAHs) are the primary toxic constituents of crude oil and have been shown to induce cardiac dysfunction and vision impairment in early life stage (ELS) fishes. Because cardiac dysfunction may influence eye development, it is currently unknown whether these visual impairments are independent effects or if they result from underlying PAH cardiotoxicity. To better understand this mechanism, microinjection of the microRNA mimic 133b (miR-133b) was used to impair cardiac development in ELS zebrafish. Vision and cardiac endpoints were compared between miR-133b zebrafish larvae and oil-exposed zebrafish larvae to determine the influence of cardiac dysfunction and oil exposure on visual function. Embryonic zebrafish were exposed to weathered crude oil for 72 h. At 7 dpf, eye area was measured and visual function was assessed by optokinetic response (OKR). To evaluate cardiotoxicity, pericardial area was measured and the incidence of pericardial edema was determined. Across treatments, there was a strong negative linear correlation between pericardial area and eye area. In both the miR-133b and oil groups, OKR was significantly reduced in larvae exhibiting pericardial edema and in larvae exhibiting reduced eye area. Because pericardial edema and reduced eye area co-occurred, a negative binomial generalized linear model (GLM) was used to evaluate these two parameters separately as significant predictors of OKR. These results indicated that reduced eye area ($p < 0.0001$) rather than pericardial edema ($p=0.76$) was a significant predictor of OKR. Together, these results suggest that visual effects may result from an interaction of cardiotoxicity and other factors.

Keywords: Aquatic Toxicology, Environmental and Wildlife Toxicology

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Toxicological Effects of Organic Contaminants in Hawksbill Sea Turtle Skin Cell Cultures

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Due to the critically endangered status of the hawksbill sea turtle, in vivo research is not possible which is why in vitro research is an excellent way to gain information regarding protected species. There is limited research on the impact of persistent organic pollutants to sea turtle species and this in vitro study aims to fill some of the knowledge gaps. This study examined the cytotoxicity of the following organic contaminants: crude oil, Corexit™9500A, perfluorooctanoic acid (PFOA), polychlorinated biphenyl (PCB77), and benzo[a]pyrene (B[a]P). Cells were dosed with either (1) media accommodated fractions of crude oil and/or Corexit™9500A, (2) PFOA in concentrations of 500 µM, 50 µM, 5 µM, 0.5 µM and 0.05 µM, or (3) PCB77 or B[a]P in concentrations of 10µM, 1µM, 0.1µM, or 0.01µM. Cytotoxicity was examined via both MTT (methylthiazolyldiphenyl-tetrazolium bromide) and LDH (Lactate Dehydrogenase) assays at 24h and 96h. Results indicate that there is greater toxicity at the 96h time point and for higher concentrations of PFOA and PCB77. Regarding the media accommodated fractions, results indicate that there is significant toxicity in response to Corexit™9500A. Significant toxicity was seen after exposure to the 10µM dose of B[a]P after a 96hour exposure period. Further analyses is underway and cytotoxicity observed via MTT and LDH assays will be compared to (1) assess which assay may be more suitable for each contaminant and (2) determine the relative sensitivity of Hawksbill skin cells to the panel of contaminants tested.

Keywords: Environmental and Wildlife Toxicology

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Effects of embryonic exposure to Aroclor 1254 on neurologic and cardiac endpoints in zebrafish (Danio rerio)

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Polychlorinated biphenyls (PCBs) are synthetic organic compounds known for their toxicity to both humans and the environment. PCBs were generally released as mixtures into the environment rather than individual congeners. One such mixture, Aroclor 1254, has been reported to have the highest proportion of dioxin-like congeners. In the present study, we investigate the cardiac and neurological effects of exposure to Aroclor 1254 in zebrafish embryos. Embryos were exposed at 6 hpf via aqueous solution for 96 hr without renewal. Many studies only report nominal concentrations of Aroclor 1254; these range from 6% to 700% of actual concentrations in tissues or solutions. For this study, exposure solutions and tissue samples were analyzed for individual congeners from which total PCBs and dioxin-like toxic equivalencies were calculated. Samples for RNA-Seq analysis were collected at 102 hpf and cardiac edema was assessed. Heart rate and a neurological endpoint (eye tremors) were measured at both 102 and 174 hpf. Cardiac edema was not present in Aroclor 1254-treated zebrafish at any exposure concentration but was observed in those exposed to PCB-126 as a positive control. However, dose dependent bradycardia was observed in zebrafish exposed to Aroclor 1254 and PCB-126 at both 102 and 1174 hpf. Similarly, a dose dependent increase in eye tremor behavior, duration, and intensity was observed in embryos exposed to Aroclor 1254 at both 102 and 174 hpf. Although dopamine was not measured, eye tremor behavior appears similar to that of other dopaminergic-related neurodegeneration associated with PCB exposure.

Keywords: Aquatic Toxicology

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Effects in vitro of 20-hydroxyecdysone and chrysene on hepatopancreatic expression of a CYP4 gene in the blue crab, Callinectes sapidus

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Petroleum production in the northern Gulf of Mexico subjects marine organisms to pollution. In order to obtain a molecular biomarker for polycyclic aromatic hydrocarbon (PAH) contamination, the cytochrome P450 (CYP) system in the blue crab, Callinectes sapidus, was explored using a degenerate primer approach. A partial CYP4 cDNA sequence from the hepatopancreas, consisting of 412 bp with an open reading frame of 136 amino acids, was acquired. CYP enzymes metabolize hormones, like ecdysteroids, and xenobiotics, like PAHs. Responsiveness of the CYP4 gene to 20-hydroxyecdysone (20E), molting hormone, and chrysene, a PAH, was determined using tissue culture and quantitative real-time PCR. Hepatopancreatic tissues were exposed to three concentrations of each 20E, encompassing concentrations found during the C. sapidus molting cycle, and chrysene. CYP4 gene expression decreased with increasing 20E concentrations; 10 and 100 nM 20E significantly increased the relative abundance of CYP4 mRNA while 1 μM 20E resulted in the absence of upregulation of CYP4 expression. Such a pattern in CYP4 gene expression is consistent with previous findings that peak microsomal ethoxyresorufin O-deethylase (EROD) activity in Uca pugilator hepatopancreas occurs in postmolt when hemolymph ecdysteroid titers are depressed. Increasing concentrations of chrysene led to decreasing CYP4 mRNA amounts; chrysene at 0.2, 2 and 20 μM all significantly suppressing CYP4 gene expression. Chrysene's inhibitory effect on CYP4 mRNA in cultured hepatopancreatic tissues may indicate induced cytotoxicity. Overall, the CYP4 gene in the present study appears to be involved in ecdysteroid regulation and not suitable as a biomarker for petroleum contamination.

Keywords: Aquatic Toxicology, Environmental and Wildlife Toxicology

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Reduced phase I biotransformation of polycyclic aromatic hydrocarbons (PAHs) in pollution-adapted Gulf killifish (Fundulus grandis)

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Anthropogenic pollution represents a significant source of selection, potentially leading to the emergence of evolutionary adaptations in chronically exposed organisms. This is the case for Gulf killifish (Fundulus grandis) populations inhabiting the Houston Ship Channel (HSC), Texas, which have been documented to be pollution-adapted. Although not fully elucidated, one particularly important aspect of their adaptation involves the reduced inducibility of the aryl hydrocarbon receptor (AhR) and, potentially, the alteration of other major biotransformation pathways. To further explore how biotransformation adaptations have altered how F. grandis cope with pollutants ubiquitous in the HSC, the present study employed a modified Organization for Economic Cooperation and Development (OECD) 319B test guideline to describe intraspecific and sex-related differences in the hepatic clearance of six polycyclic aromatic hydrocarbons (PAHs) by F. grandis populations with different evolutionary and exposure histories. Pollution-adapted F. grandis showed significantly lower hepatic clearance than non-adapted fish, especially for high molecular weight PAHs. The characterization of phase I biotransformation enzymes revealed that the basal activity of CYP1A, fundamental in the biotransformation of PAHs, was significantly lower in pollution-adapted fish. Among sexes, pollution-adapted females showed the lowest hepatic clearance and CYP1A activity. These results demonstrate the importance of exposure and evolutionary histories in shaping organisms' responses to pollution and highlight the S9 substrate depletion approach as a reliable technique to support field assessments while reducing animal use.

Keywords: Aquatic Toxicology, Biotransformation