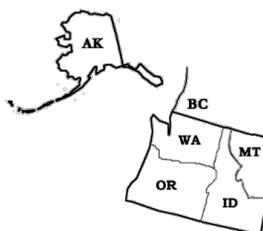




Precision Pharmacology and Toxicology

Regional Chapter Meeting
October 2-3rd, 2022





PACIFIC NORTHWEST Association of Toxicologists

2022 Regional Meeting Agenda Precision Pharmacology and Toxicology

Monday, October 3rd

8:00 - 9:00 AM	Presenters upload slides to conference computer and poster setup
8:30 - 9:00 AM	Coffee and Breakfast Pastries
9:00 - 9:10 AM	Welcome Remarks and Conference Overview <i>Dr. John Clarke, PANWAT Vice President, Washington State University</i>
9:10 - 10:00 AM	Keynote Presentation by Dr. Gary Miller The exposome and toxicology: populations to molecules <i>Moderator: Dr. Chris Carosino, PANWAT President, Seagen</i>
10:00 - 10:50 AM	Keynote Presentation by Dr. Nathan Cherrington Piggybacking the Mechanisms for Adverse Drug Reactions to Diagnose Patients with NASH <i>Moderator: Dr. John Clarke, PANWAT Vice President, Washington State University</i>
10:50 - 11:00 AM	Break
11:00 - 11:50 AM	Graduate Student Presentations: Session 1 <i>Moderator: Dr. Kari Gather, PANWAT Outreach Coordinator, PNNL</i> <i>Victoria Colvin</i> Metabolic Competency of an Airway Organotypic Culture Model <i>James Nguyen</i> Elucidating mechanisms underlying a pharmacokinetic natural product-drug interaction using a modeling and simulation approach <i>Joe Lim</i> Single cell hepatic transcriptomics revealed the role of the gut microbiome in regulating the immune-metabolic capacity of mouse liver <i>Aarzo Thakur</i> Rat as a model for predicting human renal organic anion transporter-mediated drug-drug interactions
11:50—12:15 PM	Undergraduate Student Presentations <i>Moderator: Christian Rude, PANWAT Graduate Student Representative, Oregon State University</i> <i>Madeleine Koegler</i> Implementation of Bionomous EggSorter with AI Directed Fluidic Sorting Zebrafish Embryos for High Throughput Screening <i>Francesca Rossi</i> Validation of an LC-MS/MS method for quantification of fumonisins in garlic

12:15—12:45 PM	Lunch provided
12:45 – 2:00	Poster viewing
2:00 - 2:40 PM	Graduate Student Presentations: Session 2 <i>Moderator: Dr. Lisa Truong, PANWAT Vice President elect, Oregon State University</i> <i>Victoria Oyanna</i> Green tea-raloxifene pharmacokinetic interaction: Decrease in raloxifene solubility and systemic exposure <i>Anish Mahadeo</i> Mechanistic Analysis of Ochratoxin-A Induced Nephrotoxicity <i>Rakshit Tanna</i> Clinical assessment of the drug interaction potential of the psychotropic natural product kratom
2:40 – 3:30 PM	Postdoctoral Presentations <i>Moderator: Dr. John Clarke, PANWAT Vice President, Washington State University</i> <i>Jessica Ray</i> Estrogen and cholesterol efflux regulate macrophage phenotype development <i>Hao Wang</i> Cadmium induced gut dysbiosis preceding the onset of memory deficits in mice <i>Lindsey St. Mary</i> Comparative Analysis Between Zebrafish and an Automated Live-Cell Assay to Assess 87 Developmental Neurotoxicants
3:30 - 3:40 PM	Break
3:40 - 4:40 PM	2022 PANWAT Achievement Award Lecture – Dr. Brian Thrall Key Events in Nano-Bio Interactions <i>Moderator: Dr. Haley Neff-LaFord, previous PANWAT President, Seagen</i>
4:40 – 4:55 PM	Award winners and PANWAT Endowment Awards <i>Dr. Lisa Truong, PANWAT Vice President elect, Oregon State University</i>
4:55 – 5:00 PM	Closing remarks <i>Dr. Chris Carosino, PANWAT President, Seagen</i>

Keynote Speaker

Gary W. Miller, PhD

*Vice Dean for Research Strategy and Innovation
Professor of Environmental Health Sciences
Mailman School of Public Health
Columbia University*



Dr. Miller serves as Vice Dean for Research Strategy and Innovation and Professor of Environmental Health Sciences in the Mailman School of Public Health, and Professor of Molecular Pharmacology and Therapeutics in the Vagelos College of Physicians and Surgeons at Columbia University in New York. He completed his PhD in Pharmacology and Toxicology at the University of Georgia and postdoctoral training in Molecular Neuroscience at Emory University and Duke University. His laboratory studies the role of environmental factors in neurodegenerative diseases, including Parkinson's disease and Alzheimer's disease. His research utilizes *C. elegans*, transgenic mouse models, and human studies using a variety of techniques. He is an international leader in the field of exposomics. Dr. Miller founded the first exposome center in the U.S. and wrote the first book on the topic, *The Exposome: A Primer*. In 2020 he published an updated and expanded version of the book, *The Exposome: a New Paradigm for the Environment and Health* published by Elsevier Academic Press. He has helped develop high-resolution mass spectrometry methods to provide an omic-scale analysis of the human exposome. Dr. Miller directs the Exposomics Laboratory at Columbia University, which supports the integration of environmental measures into clinical and translational research projects. He is a member of the National Institutes of Health All of Us Research Program Advisory Panel and the National Institute of Environmental Health Sciences Advisory Council, and served on the External Advisory Panel of the Human Biomonitoring for the European Union (HBM4EU) project. Dr. Miller served as Editor-in-Chief of *Toxicological Sciences*, the flagship journal of the Society of Toxicology from 2013-2019. He is the founding editor of the new journal *Exposome*, published by Oxford University Press.

Keynote Speaker

Nathan J. Cherrington, PhD

Professor of Pharmacology and Toxicology

Associate Dean for Research

Director of the Southwest Environmental Health Sciences Center

Director of the Center of Toxicology

1885 Society Distinguished Scholar

University of Arizona



Dr. Cherrington is a Professor and 1885 Society Distinguished Scholar in the Department of Pharmacology and Toxicology at the University of Arizona. He is the Associate Dean for Research in the R. Ken Coit College of Pharmacy, Director of the Southwest Environmental Health Sciences Center, and Interim Director of the Arizona Board of Regents Center for Toxicology. He received a B.S. in Zoology from Brigham Young University and a Ph.D. in Toxicology from North Carolina State University with an emphasis on xenobiotic metabolism. He then moved to the University of Kansas Medical Center to pursue postdoctoral training in drug metabolism and disposition. He has taught Drug Metabolism and Disposition, Systems Toxicology, Environmental Health Science, and Advanced Toxicology courses since joining the faculty at the University of Arizona in 2002. Nathan has published over 120 original research papers on the sources of inter-individual variability in drug response. He serves as an associate editor for *Toxicological Sciences*, and on the editorial board of *Drug Metabolism and Disposition*. He has served on numerous NIH study sections including chair of the NIEHS Environmental Health Sciences Review Committee and Severe Adverse Drug Reactions panel, as well as several committees for the Society of Toxicology and the International Society for the Study of Xenobiotics. He was awarded the Alumni Achievement Award from Brigham Young University and the Achievement Award by the Society of Toxicology. His current research is on the effect of underlying disease states on an individual's ability to metabolize and eliminate drugs.

2022 Toxicology Achievement Award

Brian Thrall, PhD

Scientist Emeritus

Pacific Northwest National Laboratory



Dr. Brian Thrall has more than 30 years of experience in toxicology and has decades of contribution in leadership, scholarship, mentoring, and service to the field and the region. He received his BS and MS in Biology from the University of South Dakota and his PhD in Pharmacology and Toxicology from Washington State University. He joined the Pacific Northwest National Laboratory (PNNL) as a research scientist three years after earning his PhD and remained there throughout his career. He became an integral part of the PNNL leadership by serving in multiple leadership capacities, including Director of the Center for Nanotoxicology and Manager of the Biology Research Sectors where he managed a \$38 million research portfolio from NIH and DOE. Brian has published a number of outstanding manuscripts in the area of mass spectrometry, gene expression, proteomics, multi-omics, and nanotoxicology. He has authored more than 100 peer-reviewed abstract and publications. Over the past 20 years he emerged as a thought leader in the global effort to understand the toxicity and risk posed by engineered nanomaterials. His leadership at the National Institutes of Environmental Health Sciences was instrumental to drive this field forward. He organized and participated in dozens of symposia and workshops at local, regional, national and international venues advancing numerous disciplines. Dr. Thrall is a past President of PANWAT (2000-2001) and has a long list of awards for his scholarship and leadership accomplishments. Although he was not at an academic institution, he has an impressive list of trainees who have gone onto successful careers of their own. Congratulations to Dr. Thrall for earning the 2022 PANWAT Toxicology Achievement Award for his substantial contributions to toxicology in the Pacific Northwest and throughout the world.

Graduate Student Presentation Abstracts: Session 1

Metabolic Competency of an Airway Organotypic Culture Model

Victoria Colvin, Kelley Bastin, Lisbeth K. Siddens, Monica Maier, Norah Owiti, Bevin Engelward, David E. Williams, Susan C. Tilton

Oregon State University

Polycyclic aromatic hydrocarbons (PAHs) are formed during the incomplete combustion of organic materials and some have been associated with several forms of cancer, including lung. PAHs are bioactivated into reactive metabolites by metabolizing enzymes in the body to cause mutations and altered gene signaling leading to tumor growth. The airway epithelium is a primary route of exposure for inhaled toxicants, and 3D organotypic culture models represent an important advancement for toxicity testing compared to traditional in vitro models that lack metabolic capability and multicellular structure/communication associated with the bronchial epithelium in vivo. However, limited data exists regarding the metabolic capacity of these models, which limits their use in quantitative studies for assessing dosimetry or predictive modeling of toxicity compared to in vivo studies. Therefore, primary human bronchial epithelial cells (HBECs) cultured at the air-liquid interface were utilized as a model for PAH inhalation toxicity. A number of comparative approaches have been utilized to assess the metabolic competency of HBECs after treatment with benzo[a]pyrene (BaP, 0.5-50 µg/mL) including 1) benchmark modeling describing significant effects on DNA damage, xenobiotic response, and oxidative stress pathways 2) qPCR confirming dose-dependent changes for several Phase I and II enzymes 3) UPLC analysis showing the formation of many BaP metabolites 4) P450-glo activity data confirming increased CYP1A1 activity and 5) CometChip analysis demonstrating BaP metabolite-induced DNA damage. Overall, this study helps to demonstrate the relevance of in vitro 3D primary culture models for chemical toxicity evaluation in the lung.

Graduate Student Presentation Abstracts: Session 1

Elucidating mechanisms underlying a pharmacokinetic natural product-drug interaction using a modeling and simulation approach

James T. Nguyen, Rakshit S. Tanna, Dandan Tian, Chris Arian, Nadja B. Cech, Nicholas H. Oberlies, Allan E. Rettie, Kenneth E. Thummel, Mary F. Paine

Department of Pharmaceutical Sciences, Washington State University

Background. The herbal product goldenseal is an inhibitor of cytochrome P450 (CYP) 3A activity, as evidenced by an approximately 50% increase in the area under the plasma concentration versus time curve (AUC) of the probe drug midazolam after oral administration with goldenseal. The collective in vitro and clinical pharmacokinetic studies prompted development of a physiologically based pharmacokinetic (PBPK) model to 1) predict plasma exposure to the major goldenseal alkaloids berberine and (-)- β -hydrastine, 2) distinguish the contributions of reversible and time-dependent CYP3A inhibition by each alkaloid, and 3) determine the primary anatomical site of the interaction. **Methods.** A PBPK inhibitor model was developed for each alkaloid using Simcyp™. Physicochemical properties, CYP3A inhibition kinetics, and clinical pharmacokinetic data obtained from the literature were incorporated into the models. The developed inhibitor models were combined with the midazolam substrate model to simulate alkaloid exposure and predict the magnitude of the goldenseal-midazolam interaction. **Results.** The predicted midazolam AUC_{0-12h} ratio (AUC in presence to absence of goldenseal) after multi-day goldenseal administration was within 10% of the observed ratio (1.48 versus 1.39). Simulations demonstrated that goldenseal maximally decreased active CYP3A enzyme in the gut and liver by ~70% and ~10%, respectively. Removing either the (-)- β -hydrastine model or time-dependent inhibition from the simulation resulted in a predicted <5% decrease in active CYP3A enzyme in the gut and liver and no change in midazolam AUC. **Conclusions.** Simulations implicated (-)- β -hydrastine as the major phytoconstituent that precipitates the observed pharmacokinetic goldenseal-midazolam interaction, primarily by inhibiting gut CYP3A via time-dependent inhibition. PBPK modeling and simulation represent a robust technique that can be used to elucidate mechanisms underlying other natural product-drug interactions.

Graduate Student Presentation Abstracts: Session 1

Single cell hepatic transcriptomics revealed the role of the gut microbiome in regulating the immune-metabolic capacity of mouse liver

Joe Lim and Julia Yue Cui

Department of Environmental and Occupational Health Sciences, University of Washington

The liver is a heterogeneous organ, containing multiple cell types for xenobiotic biotransformation, nutrient homeostasis, and immunological functions. Resident liver cells include hepatocytes, cholangiocytes, Kupffer cells, stellate cells and endothelial cells, along with circulating leukocytes. Emerging research shows that the gut-liver axis critically regulates hepatic functions. Using single cell transcriptomics, we tested our hypothesis that the presence of gut microbiota critically impacts cell type-specific signaling pathways in the liver. We developed an enzymatic whole liver dissociation method, which was applied to obtain liver cells from 7-8 month old adult male conventional (CV) and germ-free (GF) C57BL/6 mice. The dissociated cells were subject to single cell RNA-sequencing in triplicates. In GF liver, the relative hepatic proportions of cholangiocytes, stellate cells, B cells, and natural killer cells increased, but the proportion of endothelial cells, macrophages, and neutrophils decreased. In hepatocytes, the absence of microbiome upregulated the mRNA expression of cytochrome P450 (Cyp) 2b10, a prototypical target gene of the xenobiotic-sensing nuclear receptor constitutive androstane receptor (CAR). Subclustering of cell types showed a greater proportion of immuno-active hepatocytes and macrophages in livers of GF mice. Ligand-receptor communication analysis showed that pro-inflammatory complement communication were increased in hepatocytes and anti-inflammatory galectin pathways were decreased in Kupffer cells of GF mice, indicating that the presence of gut microbiome primes the liver to be more immunotolerant. Overall, our results suggest that the gut microbiome is a key modulator of xenobiotic biotransformation and lipid oxidation. Together with insights on understanding the impact of the gut microbiome on the constitutive immune functions in multiple cell types in the liver, these results will help understand the pathogenesis of various liver diseases.

Graduate Student Presentation Abstracts: Session 1

Rat as a model for predicting human renal organic anion transporter-mediated drug-drug interactions

Aarzoo Thakur, Vijay Mettu, James T. Nguyen, Mary F. Paine, and Bhagwat Prasad

Department of Pharmaceutical Sciences, Washington State University

The use of endogenous metabolites as transporter biomarkers to predict transporter-mediated drug-drug interactions (DDIs) is emerging. Organic anion transporters (OAT/Oat1/3) facilitate the uptake of several drugs and endogenous metabolites for renal secretion. Inhibition of OAT1/3 by probenecid (PROB) in human participants has been associated with elevated plasma concentrations of various endogenous metabolites, including pyridoxic acid (PDA) and kynurenic acid (KNA). The objective of this study was to evaluate whether changes in putative Oat biomarkers in rat blood and urine due to transporter inhibition can be extrapolated to OAT activity in humans. We conducted a pharmacokinetic DDI study in rats ($n=6$) involving the OAT/Oat substrate furosemide (FSM) (5 mg/kg i.v.) and inhibitor PROB (10 mg/kg i.v.). Blood and urine were collected from 0-8 h and analyzed for FSM, PROB, PDA, KNA, and creatinine (CREAT) by liquid-chromatography tandem mass spectrometry (LC/MS/MS). In the presence of PROB, at 1 h, FSM blood concentration correlated with PDA ($r=0.98$) and KNA ($r=0.89$) concentration, suggesting the utility of PDA or KNA blood concentrations to predict changes in FSM (or potentially other drug substrate) blood concentrations upon Oat inhibition. Average renal clearance (CL_{renal}) ratio for FSM, PDA, KNA, and CREAT was 0.39, 0.75, 0.79, and 0.96, respectively. The average CL_{renal} ratio for FSM, PDA, KNA, and CREAT measured in two human participants from an ongoing clinical PROB-FSM DDI study were 0.38, 0.55, 0.71, and 0.99, respectively, consistent with the rat data. The similar CL_{renal} ratios between rats and humans suggest that the rat may be a useful model to predict OAT-mediated DDIs in humans. If these trends hold for all 16 participants in this powered clinical study, results from the controlled rat study could be used to identify a panel of metabolites as OAT biomarkers, which in turn would normalize the inter-individual variability in one or more biomarkers.

Undergraduate Student Presentation Abstracts

Validation of an LC-MS/MS method for quantification of fumonisins in garlic

Francesca Rossi¹, Jeremiah Dung², Khuong Hua², Jennifer Durringer¹

Oregon State University, ¹Environmental & Molecular Toxicology, ²Botany & Plant Pathology

Fumonisin is a group of mycotoxins produced by the fungus *Fusarium proliferatum* that are known for their toxicity to mammalian herbivores. In humans, toxicity targets the liver and kidneys, as well as causes abnormalities in fetal development. The food safety threat of fumonisins affects several crops, including corn, wheat, sorghum, asparagus and, more recently, garlic. Manifesting as “garlic rot,” fumonisin contamination of garlic poses a severe economic threat, as garlic is grown in nearly every region of the world. The aim of this project is to validate a method previously used in peanut and corn matrices for use in detecting fumonisin B1 and B2 in garlic. Linearity and selectivity were established: R² values of >0.98 were obtained and both compounds were successfully separated via LC-MS/MS. Method sensitivity values included limits of detection and quantification of 1.4/4.1 ng/mL for fumonisin B1 and B2, respectively. Store-bought garlic powder was extracted and verified as a blank matrix. Matrix effects were measured by comparing peak area of neat standards to that of spiked blank extract. Additional validation steps to be completed include recovery, accuracy (evaluation of real samples) and development of quality control materials. This fully validated method will allow for analysis of samples from a project which aims to assess the genetic diversity of *Fusarium proliferatum* within USDA garlic cultivars and pair that with fumonisin production, with the ultimate goal being development of management strategies to minimize economic losses for garlic farmers due to *Fusarium proliferatum* infection.

Undergraduate Student Presentation Abstracts

Implementation of Bionomous EggSorter with AI Directed Fluidic Sorting Zebrafish Embryos for High Throughput Screening

Madeleine Koegler, Michael Simonich, Lisa Truong, Joshua White, Robyn L. Tanguay

Sinnhuber Aquatic Research Laboratory, Department Environmental and Molecular Toxicology, Oregon State University

The Bionomous EggSorter (<https://bionomous.ch/bioeggssorter/>) recently entered the market as a user-friendly and affordable beta platform to address limitations around manual egg sorting and staging. The core of the design is trainable AI classification algorithms, an optical path and a sorting wheel. We sought to evaluate the EggSorter in a real world, high throughput setting for its ability to 1) distinguish viable and fertilized eggs, 2) its accuracy in selecting target age stages of 2.5 and 6 hpf and 3) its accuracy and speed in singulating eggs into 96 well plates, without damage. Our results show that the classification algorithms are not yet fully optimized. Classification of the 2.5 hpf target from a mixture ranging from 1.5 – 3.0 hpf was accurate approximately 40% of the time. The need for more algorithm training on this target stage was thus apparent. Embryos near the 2.5 hpf target also experienced an unacceptable incidence of damage by the sorting process, apparently in the wells of the sorter wheel itself. It is hoped that a cleaner-machining material will be superior to the current plastic wheel. Classification of the 6 hpf target from a mixture of stages from 4.5 – 10 hpf was accurate better than 90% of time, suggesting that the algorithm training was nearly sufficient for this target. The time required to load a 96 well plate at the 6 hpf target was approximately 15 minutes and no instance of a well left empty was observed for the 6 hpf target. Double loading of wells ranged from 2 – 4 wells per plate and may be mitigated by reducing the density of eggs in the supply hopper. The 6 hpf target embryos were free of damage. The goal of the EggSorter is to provide an efficient process of sorting embryos that enables the researcher to focus on more technical tasks. More algorithm training on earlier targets and minor hardware improvements will effectively automate all aspects of zebrafish egg sorting, staging and allocation.

Graduate Student Presentation Abstracts: Session 2

Green tea-raloxifene pharmacokinetic interaction: Decrease in raloxifene solubility and systemic exposure

Victoria O. Oyanna and John D. Clarke

Department of Pharmaceutical Sciences, Washington State University

Green tea is a popular botanical natural product, and its consumption has been associated with pharmacokinetic natural product-drug interactions (NP-DIs). Unpublished clinical data show that acute and chronic oral administration of green tea decreased the systemic exposure of raloxifene without affecting its terminal half-life, suggesting decreased intestinal absorption. The mechanism for this NP-DI is currently unknown. Raloxifene is a poorly water-soluble drug, and inhibition of intestinal uptake transporters does not influence its absorption. We hypothesize that green tea reduces intestinal absorption of raloxifene through decreased solubility. To investigate this NP-DI, fasted state intestinal fluid (FaSSIF) was incubated with raloxifene alone and in combination with varying concentrations of green tea extract (GTE) and its major catechins: (-)-epigallocatechin gallate (EGCG) and epigallocatechin (EGC). GTE (≥ 1.69 mg/ml) and EGCG (≥ 0.24 mM) decreased raloxifene solubility (20-80%), while EGC (≥ 0.3 mM) had minimal effects on raloxifene's solubility. These data indicate that catechins with a gallate moiety may be responsible for raloxifene's decreased solubility. Next, we performed an oral gavage pharmacokinetic interaction study in C57BL/6 mice, investigating the effect of GTE and EGCG on raloxifene's systemic exposure. Plasma was collected over 24 hours and analyzed for raloxifene and its glucuronide metabolites. The pharmacokinetics were determined via noncompartmental analysis using Phoenix WinNonlin. GTE decreased the maximum observed concentration (C_{max}) of raloxifene and raloxifene-6-glucuronide by 40% and 58%, respectively. Although GTE and EGCG decreased raloxifene solubility in vitro, EGCG alone did not precipitate the interaction in vivo, suggesting a mixture of catechins is required to precipitate the interaction. These data suggest that decreased solubility of raloxifene contributes to the observed NP-DI. Funding: U54 AT008909 and R21 AT011101

Graduate Student Presentation Abstracts: Session 2

Mechanistic Analysis of Ochratoxin-A Induced Nephrotoxicity

Anish Mahadeo, Dr. Edward Kelly, Dr. Catherine Yeung

University of Washington, Department of Pharmaceutics

While diabetes, hypertension, and obesity are known risk factors for CKD, over the last 50 years, endemic hotspots of chronic kidney disease around the world are thought to have environmental causes, including ochratoxin-A (OTA). Based on previous work, it is hypothesized that OTA may hinder the nuclear translocation of antioxidant-regulator NRF2 from its cytosolic binder KEAP1, thus downregulating numerous oxidative-stress responses transcribed by NRF2. To investigate this mechanism, primary human proximal tubule epithelial cells (hPTECs) were cultured with NRF2 activators sulforaphane (SFN) or tert-butylhydroquinone (tBHQ) to induce antioxidant response genes before being challenged with OTA. Total mRNA sequencing and immunocytochemistry staining for downstream NRF2 targets were utilized for assessment. RNA-seq analysis revealed numerous differentially expressed genes between cells treated with OTA, OTA + NRF2 activators, and control media. Several NRF2 targets such as GSR and GSTP1 were found to be downregulated due to OTA, even in the presence of NRF2 activators. Interestingly, several genes involved in the hypoxic response such as HIF1 α were upregulated. Immunocytochemistry analysis of cells cultured under hypoxic conditions with OTA and NRF2 activators revealed significant HIF1 α nuclear localization and cell death compared controls. This suggests a synergistic effect between OTA, the NRF2 pathway, and the hypoxic response pathway may be involved in OTA nephrotoxicity. Future experiments will investigate these processes in 3-D proximal tubule microphysiological systems, which have been demonstrated to replicate in vivo processes and microenvironment not seen in traditional cell culture. Furthermore, the role of OTA, hypoxic and oxidative stress on mitochondria will be assessed by live confocal imaging of hPTECs. To investigate the role of OTA in CKDu, plasma and urine samples from CKDu patients in Sri Lanka will be analyzed by LC-MS/MS for the presence of OTA.

Graduate Student Presentation Abstracts: Session 2

Clinical assessment of the drug interaction potential of the psychotropic natural product kratom

Rakshit S. Tanna¹, James T. Nguyen¹, Deena L. Hadi^{1,2}, Matthew E. Layton³, John R. White⁴, Nadja B. Cech^{2,5}, Nicholas H. Oberlies^{2,5}, Allan E. Rettie^{2,6}, Kenneth E. Thummel^{2,7}, and Mary F. Paine^{1,2}

¹Department of Pharmaceutical Sciences, College of Pharmacy and Pharmaceutical Sciences, Washington State University, Spokane, Washington, USA; ²Center of Excellence for Natural Product Drug Interaction Research, Spokane, Washington, USA; ³Elson S. Floyd College of Medicine, Washington State University, Spokane, Washington, USA; ⁴Department of Pharmacotherapy, College of Pharmacy and Pharmaceutical Sciences, Washington State University, Spokane, Washington, USA; ⁵Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, North Carolina, USA; Departments of ⁶Medicinal Chemistry and ⁷Pharmaceutics, School of Pharmacy, University of Washington, Seattle, Washington, USA

Background. Oral supplements from the leaves of the kratom (*Mitragyna speciosa*) plant are increasingly used for their opioid-like effects to self-manage opioid withdrawal and pain. Calls to US poison centers involving kratom exposures increased 52-fold from 2011-2017, one-third of which reported use of kratom with drugs of abuse. Many of these drugs are eliminated from the body through extensive metabolism by the cytochrome P450 (CYP) enzymes, particularly CYP2D6 and CYP3A, raising concerns for potential adverse kratom-drug interactions. The objective of this work was to conduct a powered clinical pharmacokinetic kratom-drug interaction study using a well-characterized kratom product and the probe drug substrates dextromethorphan (CYP2D6) and midazolam (CYP3A). **Methods.** Twelve healthy adult volunteers participated in an open label, two-arm crossover, fixed sequence study. Midazolam (2.5 mg) and dextromethorphan (30 mg) were administered orally with water to obtain baseline pharmacokinetics. At least one week later, participants were administered the probe drugs 15 min after consuming a low dose (2 g) of the kratom product as a tea. Plasma was collected (0-24 h) and analyzed for the probe drugs using LC-MS/MS. Pharmacokinetics were characterized via noncompartmental analysis. The geometric mean ratio (90% confidence interval) of the area under plasma concentration-time curve (AUC) and maximum plasma concentration (C_{max}) in the presence to absence of kratom were determined. **Results.** Kratom showed no effect on dextromethorphan AUC and C_{max} ratios but a modest increase in corresponding midazolam ratios [1.38 (1.23-1.57) and 1.50 (1.32-1.70), respectively]. Lack of change in midazolam half-life [1.07 (0.98-1.17)] suggested that kratom primarily inhibited intestinal CYP3A. **Conclusions.** This direct clinical evidence suggests that co-consuming kratom with other drugs extensively metabolized by CYP3A may precipitate serious interactions.

Postdoctoral Fellow Presentation Abstracts

Estrogen and cholesterol efflux regulate macrophage phenotype development

Jessica L. Ray, Rebekah L. Kendall, Britten Postma, and Andrij Holian

University of Montana

Our laboratory has previously demonstrated that following multi-walled carbon nanotube (MWCNT) exposure, female mice develop greater M2a-type inflammatory signaling by alveolar macrophages (AMs) compared to males. However, the mechanism responsible for this disparity in M2a phenotype development remains unclear. Estrogen receptor alpha (ER α) and cholesterol efflux are reported to enhance the M2a phenotype, separately. However, neither pathway has yet to be fully elucidated, nor has a connection between the two been investigated. Therefore, we hypothesized that the female bias in M2a inflammatory signaling by AMs is because estrogen signaling promotes M2a phenotype development by increasing cholesterol efflux. To test this hypothesis, the ER antagonist, Fulvestrant was given in vivo. Fulvestrant, was protective against MWCNT-induced type 2 inflammation in both sexes, but to a greater degree in females compared to males. Importantly, Fulvestrant attenuated M2a phenotype development; activation of the M2a essential transcription factor STAT6 and expression of M2a-associated genes (Ccl24, Chil3, Arg1, Gata3, Il5) were significantly reduced. Next, primary AMs from male and female mice were incubated with estrogen for 48 hr then changes in cholesterol and M2a polarization assessed. Estrogen reduced cholesterol levels (promoted efflux) in female, but not male, AMs. In accordance, M2a polarization (IL-13-induced STAT6 activation) was increased by estrogen treatment in female AMs but not males. Additionally, bone marrow-derived macrophages from SR-BI KO mice, which have diminished cholesterol efflux, had reduced M2a polarization capabilities following both IL-4 and IL-13 treatment, confirming the importance of cholesterol efflux in M2a development. Taken together, these data provide foundational evidence for the contribution of estrogen-mediated regulation of cholesterol metabolism in respiratory immune cell function and subsequent impacts on sex-based outcomes in disease. NIEHS R21ES030978-01

Postdoctoral Fellow Presentation Abstracts

Cadmium induced gut dysbiosis preceding the onset of memory deficits in mice

Hao Wang¹, Zhengui Xia¹, Haiwei Gu², Julia Yue Cui¹

¹Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA; ²Center for Translational Science, Florida International University, Port St. Lucie, Florida.

Cadmium (Cd) is a heavy metal that has been recognized as one of the most toxic environmental toxicants. Increasing evidence suggests that Cd is a neurotoxicant, however, the underlying mechanisms are not completely understood. The gut-brain axis is a communication pathway between the central nervous system and the gut microbiome. Studies have found that alterations of the gut microbiome are implicated in different neurological diseases. Because the gut microbiome is a target of Cd toxicity, the gut-brain axis may mechanistically contribute to Cd-induced impairment on learning and memory. As a first attempt to investigate the role of the gut-brain axis in Cd neurotoxicity, we tested the hypothesis that Cd-induced gut dysbiosis precedes the onset of cognitive deficits. We exposed 8-week-old C57BL/6 male mice to 3 mg/L Cd through drinking water for 9 weeks. Novel Object Location test, which probes for hippocampus-dependent spatial memory deficits, was performed during the exposure period to detect the onset of cognitive deficits. Fresh fecal pellets were collected every week to examine the alteration of the microbiome over the time course. Cd caused learning and memory deficits starting at 4 weeks into exposure. Metagenomic sequencing showed that Cd-induced dysbiosis in the fecal microbiome occurred 1 week prior to the onset of cognitive deficits. Most notably, Cd exposure produced a sustained decrease of *A. muciniphila*, as well as a sustained increase of *B. thetaiotaomicron*. At the 9-weeks terminal time point, targeted metabolomics showed that Cd decreased the SCFAs butyrate and acetate in intestines and affected the levels of bile acids in serum. RNA-sequencing showed that Cd reduced the expression of genes involved in synapse function and the transport of neurotransmitters in the hippocampus. Together, our findings showed that Cd exposure induced gut dysbiosis before the memory deficits, suggesting that the gut-brain axis may contribute to Cd neurotoxicity.

Postdoctoral Fellow Presentation Abstracts

Comparative Analysis Between Zebrafish and an Automated Live-Cell Assay to Assess 87 Developmental Neurotoxicants

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The demand and ability for increased screening efficiency to assess chemical toxicity has advanced with the development of high-throughput toxicological assays involving alternative models, automation, and machine learning (ML). The objective of this study was to compare the development/neurotoxic effects of an 87-compound library in zebrafish and an in vitro high throughput cell culture system. The AsedaSciences SYSTEMETRIC® Cell Health Screen was developed to estimate human toxicity risk using supervised ML to classify acute cell stress phenotypes in HL60 cells following a 4-h exposure to a chemical of interest, while the early life stage zebrafish provides a whole systems approach to assess toxicity. In this study, chemical hits for abnormalities in zebrafish morphology, mortality, and behavior were compared with chemicals classified as high-risk according to AsedaSciences Cell Health Index (CHI™), which is an outcome class probability from the ML classifier using 12 parameters simultaneously from the cell-based screen (CHI=1: highest possible toxicity risk). Zebrafish were more sensitive, with 79 total bioactive hits, while the SYSTEMETRIC screen identified 20 chemicals as potentially toxic. The zebrafish embryo and larval photomotor response assays (EPR and LPR respectively) identified 40 of the 47 chemicals that were not identified by the zebrafish morphological and SYSTEMETRIC® Cell Health screens. We found the 20 high-CHI chemicals highly correlated with nuclear receptor, neurodevelopment, and cell cycle endpoint hits among the 1,385 different in vitro assays curated on the EPA's CompTox Dashboard. Collectively, these results illustrate the advantages of using two alternative models in tandem for rapid hazard assessments and chemical prioritization as well as the effectiveness of CHI to identify toxicity within a single multiparametric assay.

Poster Abstracts

#1 Effect of cimetidine mediated inhibition on the levels of exogenous and endogenous substrates of organic cation transporters in rats

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Kidney is one of the major drug elimination organs. The extent of renal excretion of a compound is the net result of glomerular filtration, tubular secretion, and reabsorption. Tubular secretion is a transporter mediated process, which is often mediated by organic cation transporters (OCTs/Octs) that facilitate active excretion of several cationic substrates including drugs such as metformin as well as endogenous cations. We hypothesize that administration of cimetidine, a OCT/Oct inhibitor, will lead to increased plasma levels and decreased renal clearance of endogenous OCT/Oct substrates, which can be used to predict changes in metformin pharmacokinetics. This hypothesis was tested in rats pharmacokinetic (PK) study with the following two specific aims: i) to evaluate the effect of cimetidine on metformin pharmacokinetic parameters, and ii) to study the effect of cimetidine on putative endogenous substrates of Octs by using metabolomics for rat blood and urine samples. Metformin (5 mg/kg) was administered to four Sprague-Dawley rats with and without cimetidine (100mg/kg) in cross over study design with one week washing period. The animal study design was composed of two arms; the first arm includes the four rats which were injected with metformin alone, while the second arm includes the same rats which were injected with both metformin and cimetidine. Blood samples were collected at different time points, i.e, 0(pre-dose), 0.17, 0.5, 1, 2 ,4, 6, 8 hr and urine samples were collected at 0-4 and 4-8 hr intervals. Rat blood and urine samples were first analyzed for metformin and cimetidine levels by a validated LCMS/MS method, and the effect of cimetidine on metformin blood levels and renal clearance were evaluated. Metformin area under the curve (AUC(0-8hrs)) was significantly increased by 3.2 folds in the cimetidine arm with p-value equal to 0.003. Similarly, the metformin renal clearance (Cl_r(0-8hrs)) was significantly decreased in the cimetidine arm by 3.7 folds

Poster Abstracts

#2 Identifying Novel Endogenous Substrates of the CYP1B1 Enzyme in Exosomes: Implications for Ocular Disease and Wound Repair

Anna DePaepe, Abdulaziz Alshehri, Nader Sheibani, Patrick L. Iversen, Craig B. Marcus, Manuel Garcia-Jaramillo, and Andrew J. Annalora

Oregon State University

The cytochromes P450 (CYP) superfamily of enzymes metabolize both therapeutic drugs and environmental contaminants, but also regulate cell growth and homeostasis. CYP gene expression is complex and may modulate cargo of extracellular vesicles (e.g., exosomes (50-150 nm in diameter) via a process that is currently poorly understood. Dysregulation of tissue specific CYP expression contributes to many diseases, like primary congenital glaucoma (PCG), which is linked to >150 mutations in CYP1B1. We hypothesize that CYP1B1 regulates retinal endothelial cell (REC) function via several mechanisms, including endogenous substrate metabolism, and extracellular trafficking of exosomes. The aims of my project are to: 1) purify exosomes from “conditioned” REC media; 2) validate exosome quality by monitoring the expression of key surface markers (e.g., CD9, CD63 and CD81) via western blot; 3) determine if CYP1B1 enzyme is present in REC exosomes via western blot; 4) conduct targeted oxylipin metabolomics profiling to determine CYP1B1’s role in exosome trafficking of lipid metabolites, and 5) to identify the role exosomes play in wound repair processes using REC scratch assays, and fin clip assays in zebrafish. Our group has compared several methods for isolating purified exosomes from conditioned REC media with the ultracentrifuge (U/C) and precipitation (ExoQuick TC Ultra) methods generating the most purified and monodisperse exosomes with a mean particle size near 100 nm. Total protein from REC cells has been used in western blot analysis. We have found it challenging to consistently detect diagnostic exosome markers and CYP1B1 in exosome samples purified from cell culture media. We are currently testing new methods and antibodies to improve detection. These results will clarify the role exosomes play in modulating intercellular CYP1B1-signaling cascades in REC cells, which may have therapeutic applications for glaucoma and other CYP-mediated environmental diseases.

Poster Abstracts

#3 Transcriptomic Responses Underlying Site-Specific Toxicity in Developing Zebrafish Exposed to Whole Mixtures from Portland Harbor Superfund Site Passive Sampling Extracts

Christian I. Rude, Sarah E. Allan, Susan C. Tilton, Kim A. Anderson, Katrina M. Waters, Robyn L. Tanguay

Oregon State University

The Portland Harbor Superfund Site (PHSS) is an area of active remediation along the Willamette River in Portland, Oregon. Its proximity to residential and recreational areas requires precise resolution of contaminant concentration and toxicity to ensure remediation adequately protects human health. PHSS provides a unique challenge because numerous point and nonpoint sources drive varying contaminant levels across limited spatial and temporal scales. To address this, high-density polyethylene passive sampling devices (HDPE PSDs) were deployed for 30-day periods in nine locations across six months in and around PHSS for 54 total deployments. In addition to chemical characterization of the PHSS, serial dilutions of PSD extracts were utilized in static waterborne exposures of dechorionated zebrafish from 6 – 120 hours post fertilization (hpf) to assess the toxicity of each location attributable to its bioavailable hydrophobic contaminants. Morphological assessments at 24 and 120 hpf revealed site and month-specific toxicities including edema, malformed snout, bent axes, and a rarely observed “wavy” notochord malformation. To inform the molecular response behind site specific toxicities, we conducted RNAseq on 48 hpf zebrafish exposed to 0.75% PSD extract dilutions from two sites with diverging phenotypes. While the treatments had similar differentially expressed genes (DEGs), more robust changes were observed in the more toxic treatment. A portion of the DEGs in both treatments are consistent with those seen in zebrafish exposed to polyaromatic hydrocarbons (PAHs) and oxygenated PAHs, known contaminants of the sites. We hypothesize that non-PAH-attributable DEGs arise from mixture effects or additional unidentified toxicants. This underscores the utility of pairing PSD extracts with developmental zebrafish assays for unbiased toxicity sensing in complex environmental mixtures. This research was funded by NIEHS award numbers: P42ES016465, P30ES030287, and T32ES007060.

Poster Abstracts

#4 A review of the electronic cigarettes' withdrawal severity symptoms among young adult users and non-users

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Background: The popularity of electronic cigarettes (e-cigarettes) as a smoking cessation tool has increased within the last few years with a significant increase in the United States. There is limited data on the common symptoms associated with e-cigarettes dependence among young adults. **Methods:** The results are from the review of peer-reviewed literature on e-cigarettes through a PubMed search through June 2022. The keywords included “electronic cigarette”, “young adults”, “withdrawal symptoms”. The observational studies, experimental studies, qualitative studies, intervention studies, and mixed methods studies providing empirical findings on e-cigarette marketing and communication were included. **Results:** Forty five free full texts in English were identified with topics including the withdrawal symptoms from e-cigarette abstinence among former smokers, withdrawal symptoms among current e-cigarettes users, withdrawal symptoms from e-cigarette abstinence among adults who were never smokers, and withdrawal symptoms in dual users of cigarette and e-cigarettes. The symptoms unique to vaping dependence included nicotine consumption due to accessibility and lack of restrictions, habitual vaping, vaping frequency not being tracked, immediate gratification and satisfaction, social acceptability and norms, and awareness of vaping dependency. **Conclusion:** The young adults have described the nicotine dependence symptoms similar to the conventional cigarette. The withdrawal symptoms can occur in the never smokers, former smokers, and the current smokers who are daily e-cigarette users and the severity of withdrawal from e-cigarettes is somewhat lesser than from the daily tobacco cigarette use. Although e-cigarettes are considered as a smoking cessation aid, it can maintain physical dependence. Thus, there is a need of regulation including the information for the current e-cigarette users about the relationship between the withdrawal symptoms with the cessation of e-cigarettes.

Poster Abstracts

#5 Knocking Out the Zebrafish CYP1B1 Gene Alters Metabolomic Profiles and Neurobehavioral Functions

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Cytochrome P450 1B1 (CYP1B1) is an enzyme responsible for the metabolism of a broad range of xenobiotic and endogenous substrates including those vital to the development of the eye. Moreover, CYP1B1 mutations have been implicated in the onset of primary congenital glaucoma (PCG). PCG alters the vasculature of the ciliary bodies and trabecular meshwork surrounding the lens leading to poor aqueous flow. The resulting increase in intraocular pressure damages the optic nerve causing vision loss. Further research examining CYP1B1's mechanistic role in PCG is required to explore novel therapeutic strategies. Previously a CYP1B1 knockout (KO) mouse retinal endothelial cell model demonstrated a loss of capillary formation and altered metabolism. CYP1B1's role in PCG was explored *in vivo* using CYP1B1-KO zebrafish by performing behavioral assays and untargeted liquid chromatography mass spectrometry-based metabolomics. KO zebrafish showed a significant differential response when compared to their wild type (WT) counterparts in multiple behavior assays performed at larval and adult life stages. During the larval photo-motor response (LPR) assay KO zebrafish displayed elevated movement in response to both light and dark stimuli. In vision driven adult behavioral assays KO zebrafish displayed differential responses to videos of both schooling zebrafish and a predator. In addition, untargeted metabolomics analysis of whole larval zebrafish revealed significant differences in nucleoside and amino acid compounds among others. This research was supported by the National Institute of Environmental Health Sciences of the National Institutes of Health under Award Number P30 ES030287 and the Agriculture Research Foundation. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Poster Abstracts

#6 Polybrominated diphenyl ether (PBDE) and its effects on specific gut microbes and their associated metabolic networks relating to cellular growth and function

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Poly-brominated diphenyl ethers (PBDEs) were formerly used flame retardants and are persistent environmental toxicants implicated in various metabolic diseases. We have previously shown that oral exposure to the diet enriched PBDE congeners 2,2,4,4-tetrabromo-diphenyl ether (BDE-47) and 2,2,4,4,5-pentabromo-diphenyl ether (BDE-99) produced gut dysbiosis in large intestinal content of mice, including a marked elevation of the short chain fatty acid (SCFA)-producing *Akkermansia muciniphila*. We also showed that BDE-99 exposure increased the DNA encoding microbial 7 α -dehydroxylation enzymes for secondary bile acid (BA) synthesis. In this study, we followed up to investigate how BDE-47 and BDE-99 directly regulate specific microbes involved in SCFA- and secondary BA-synthesis. *Akkermansia muciniphila*, *Clostridium scindens*—a well-known commensal bacteria involved in secondary BA-synthesis, were exposed to either BDE-47 or BDE-99 at the following concentrations: 0 μ (control), 10 μ M, and 100 μ M. *Escherichia coli* was exposed in parallel as a positive control. Mass spectrometry analysis was used to observe metabolomic changes. Over the time course, both BDE-47 and BDE-99 showed growth inhibition of *A. muciniphila* and *E. coli*; the growth of *C. scindens* was also inhibited by BDE-99 but was promoted by BDE-47. In *C. scindens*, BDE-47 decreased 2-pyrrolidone but increased urocanic acid, whereas BDE-99 increased lactate but decreased kynurenine. In *A. muciniphila*, BDE-47 increased NADPH while BDE-99 increased 5-aminolevulinic acid. In *E. coli*, BDE-47 increased both aspartate and 4-aminobutyric acid while BDE-99 increased both 2-pyrrolidone and glucosamine. In conclusion, our study showed that PBDEs impacted the growth as well as carbohydrate and amino acid metabolism in important intestinal commensal bacteria. Understanding the direct interactions between orally exposed environmental chemicals and gut microbes may provide additional insights into the mechanisms of toxicity.

Poster Abstracts

#7 Phenotypically-anchored RNA sequencing reveals gene profiles associated with increasing retene concentration in zebrafish

Lindsay B. Wilson, Ryan S. McClure, Katrina M. Waters, Robyn L. Tanguay

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants produced via both anthropogenic and natural sources and are associated with human disease. Retene is a frequently detected PAH in environmental sampling and has been associated with aryl hydrocarbon receptor (AHR)-dependent developmental toxicity in zebrafish. Retene induces teratogenicity at as low as 20 μM in zebrafish larvae. To interrogate mechanisms of retene toxicity at concentrations anchored to teratogenicity, we utilized RNA sequencing to identify differentially expressed genes (DEGs) across eight increasing retene concentrations. Sequencing analysis identified a concentration-response relationship between observed bioactivity and number of DEGs from 0-5 DEGs identified at concentrations with no phenotype up to 707 DEGs at 50 μM , a concentration which induces 100% morphological effects. Gene co-expression network analysis of all DEGs identified several gene modules. One, Module 7, was strongly enriched for retene-responsive DEGs including novel genes indicating mechanisms of retene response that are unique from PAH response generally. Additionally, several genes showed correlation between increasing concentration and magnitude of expression, allowing for estimation of benchmark concentrations (BMC). The average BMC10 of Module 7 genes was 7.5 μM , nearly the exact concentration at which teratogenicity was first observed. This novel approach leverages high-throughput bioactivity screening data and RNA-sequencing to identify gene profiles useful for elucidating toxicogenomic mechanisms of chemical bioactivity. Research reported here was supported by the National Institute of Environmental Health Sciences of the National Institutes of Health under Award Number P42ES016465, P30ES030287, and T32ES007060. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Poster Abstracts

#8 Comparative hazard potential of environmentally relevant alkylated PAHs

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With an increasing demand for fossil fuels and a growing frequency of wildfires, polycyclic aromatic hydrocarbons (PAHs) are an environmental contaminant of growing concern. While PAHs are typically present in complex mixtures, concern has primarily been focused on the carcinogenicity of unsubstituted PAHs. Understanding the toxicity of alkylated PAHs is important for a comprehensive understanding of the hazard potential of this diverse chemical class. 77 alkylated PAHs were screened for morphological and behavioral effects in a high-throughput developmental zebrafish assay. Embryos were exposed to 12 concentrations ranging from 0 to 100uM (n =14). Embryos were screened for morphological effects using 10 morphological endpoints and behavior using a larval photo motor response. The aryl hydrocarbon receptor (AHR) is often implicated in the toxicity of PAHs and the induction of cytochrome P4501A is an excellent biomarker of AHR activation. Embryos were evaluated for spatial Cyp1a expression patterns using an AHR-responsive reporter line. A consensus QSAR model for Ames assay prediction was used to characterize and compare the mutagenic potential of these alkylated PAHs to their toxicity classification. Of the 77 alkyl PAHs tested, morphological effects were detected in 23 and it is expected that behavior endpoints will be more sensitive. The Ames assay prediction classified 38 of the 77 PAHs with a high likelihood to be mutagenic, 16 of these also induced morphological effects. 18 of those classified as mutagenic but not captured in our morphological screening, may have been missed due to solubility-dependent concentration limitations. Research reported in this publication was supported by the NIEHS of the National Institutes of Health under Award Number P42ES016465, P30ES030287, and T32ES007060. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Poster Abstracts

#9 Silymarin reduced microcystin-LR-elicited hepatotoxicity

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Cyanobacteria, found in waters across the globe, produce a class of hepatotoxins known as microcystins (MCs). MC-leucine arginine (MC-LR) is reported to be one of the most toxic and abundant of the more than 200 congeners found to date. MC-LR undergoes hepatic uptake from the blood by organic anion transporting polypeptides (humans: “OATP”; rodents: “Oatp”) 1B1 and 1B3 in humans and 1b2 in rodents. MC-LR covalently binds to and inhibits protein phosphatases 1 (PP1) and 2A (PP2A), disrupting homeostasis of protein phosphorylation and altering many cellular processes related to cytoskeleton, redox balance, and cell survival. Previous data indicate that the natural product silymarin, an extract of milk thistle seed (*Silybum marianum* (L.)) protected against MC-LR hepatotoxicity through the antioxidant activity of its flavonolignan constituents. Silymarin flavonolignans also inhibit the same OATPs responsible for hepatic uptake of MC-LR. We hypothesize that silymarin-mediated hepatoprotection involves both inhibition of MC-LR hepatic uptake and augmented antioxidant response. Male Sprague Dawley rats were exposed to a single hepatotoxic dose of MC-LR (3 mg/kg) with or without silymarin (500 mg/kg) via gavage. Blood samples were collected over 4 hours for MC-LR toxicokinetics analysis, and livers were collected at 4 hours for toxicological assessment. Although silymarin decreased plasma alanine aminotransferase (ALT) levels compared to the MC-LR only group, no differences in MC-LR toxicokinetics was observed due to silymarin. Silymarin did not affect protein expression of PP1, PP2A, Oatp1b2, or the amount of protein bound MC-LR in the liver. Further research is required to determine whether silymarin altered hepatic antioxidant capacity and/or reduced total MC-LR hepatic exposure. Funding: National Institute of Environmental Health Sciences ES032558

Poster Abstracts

#10 Cell type specific effects of MCLR: hepatocytes and stellate cells in co-culture

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Microcystin-leucine arginine (MCLR) is one of the most toxic and widely studied forms of cyanotoxins produced by blue-green algae. MCLR is primarily hepatotoxic because it is a substrate for organic anion-transporting polypeptide uptake transporters expressed predominantly in hepatocytes. Chronic and repetitive activation of hepatic stellate cell (HSC) is a major driver for liver fibrosis, and sub-chronic MCLR exposure in rats increased expression of actin alpha-2 (ACTA2)/alpha-smooth muscle actin (α -SMA), a marker of HSC activation. HepaRG cells, an immortalized hepatic cell system with three cell types, were more sensitive to MCLR toxicity than LX-2 cells, a human HSC line. Direct activation of LX-2 cells by MCLR was not observed as determined by ACTA2 expression. We hypothesize that MCLR indirectly activates HSCs through damaged hepatocytes. Cell-type specific effects of MCLR on hepatocytes and HSCs were determined in a co-culture scenario, with HepaRG cells seeded into 6-well plates and LX-2 cells seeded into transwell inserts. Interestingly, western blot analysis of protein bound MCLR occurred in both cell types, however more protein bound MCLR bands were observed in HepaRG cells compared to LX-2 cells. Although MCLR induced cell death in HepaRG cells, canonical apoptosis pathways were not activated at 24 hours of treatment, suggesting non-canonical cell death pathways may be involved. In addition, α -SMA western blot data demonstrated no activation of LX-2 cells with up to 10 μ M MCLR in either mono- or co-culture scenario. More research is needed that incorporates additional cell types and/or direct cell to cell contact to determine the mechanism for indirect activation of HSCs by MCLR. Funding: National Institute of Environmental Health Sciences ES032558

Poster Abstracts

#11 Maternal PBDE exposure disrupts gut microbiome and liver expressions in humanized PXR-transgenic mouse offspring over a time course

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Polybrominated diphenyl ethers (PBDEs) are previously used flame retardants that still bioaccumulate in human compartments that activates pregnane X receptor (PXR). PXR and gut microbiome are essential regulators of xenobiotic biotransformation and metabolic disorders. The microbial tryptophan metabolites, indole 3-propionic acid (IPA), correlates with reduced risk of type-2 diabetes and lower grade inflammation and activate PXR. To explore the potential role of IPA on modifying the toxic effects of PBDEs during development, female humanized PXR-transgenic (hPXR-TG) mouse dams were exposed to vehicle, DE-71 (an industrial PBDE mixture) via diet, DE-71 + IPA via drinking water, or IPA, from 4-weeks preconception to the end of lactation. Male and female pups were weaned at 21-days of age and were placed on control diet with or without IPA supplement until specific time points; 21 days, 3 months, and 6 months of age (n=5/exposure/sex/age). In general, the gut microbiome of pups were amplified by maternal DE-71 exposure, as there was more differentially regulated obesity- and inflammation-prone microbes at 3 months and 6 months than 21 days. The liver mRNA expression of several pro-inflammatory markers was persistently up-regulated with an amplified trend along the developmental trajectory. *Cyp1a2* was persistently increased by maternal DE-71 in all ages of males, but not in females. *Nqo1* was up-regulated by maternal DE-71 in livers of 6 months males and females. LC-MS showed that DE-71 exposure increased the serum indole levels in males. The endogenous IPA levels was decreased by DE-71 in livers 3 months males. IPA supplementation partially normalized the related metabolic profiles described above. In conclusion, maternal DE-71 exposure produced a pro-inflammatory signature within the gut-liver axis associated with dysregulated tryptophan microbial metabolism and elevated AhR signaling in a male-predominant manner, which was partly corrected by IPA supplementation.

Poster Abstracts

#12 Understanding combined effects of PAH exposure and inflammation contributing to toxicity in an in vitro 3D respiratory model

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Oregon State University

There is increased emphasis on understanding cumulative risk from the combined effects of chemical and non-chemical stressors as it relates to public health. Recent animal studies have identified pulmonary inflammation as a possible modifier and risk factor for chemical toxicity in the lung after exposure to inhaled pollutants; however, little is known about specific interactions and potential mechanisms of action. In this study, primary human bronchial epithelial cells (HBEC) cultured in 3D at the air-liquid interface (ALI) are utilized as a physiologically relevant model to evaluate the effects of inflammation on toxicity of polycyclic aromatic hydrocarbons (PAHs), a class of contaminants generated from incomplete combustion of fossil fuels. Normal HBEC were differentiated in the presence of IL-13 for 14 days to induce a profibrotic phenotype similar to asthma. Fully differentiated normal and asthmatic phenotype HBEC were treated with benzo[a]pyrene (BaP; 1 – 40 ug/ml) or 1% DMSO/PBS vehicle at the ALI for 48 hrs. Cells were evaluated for cytotoxicity, barrier integrity, and transcriptional biomarkers of chemical metabolism and inflammation by quantitative PCR. Cells with the asthmatic phenotype treated with BaP show significantly ($p < 0.05$) increased cytotoxicity and inflammation and significantly ($p < 0.05$) decreased barrier integrity and metabolic capacity compared to normal cells. Additionally, RNA sequencing data showed that a large number of genes were uniquely significantly expressed in cells with the induced asthmatic phenotype exposed to BaP. Future studies will further explore mechanisms of toxicity from global transcriptomics. These data are the first to evaluate the role of combined environmental factors associated with inflammation from pre-existing disease and PAH exposure on pulmonary toxicity in a physiologically relevant human in vitro model.

Poster Abstracts

#13 Characterizations of Fatty Acids from *Desmodium* sp. and *Sechium edule*

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Eggs produced from poultry species are a readily available, economic source of protein for much of the global population. Enriching the nutritional profile of egg yolks allows for delivery of optimized nutrients, particularly in geographic locations where food security is a concern. This is most easily accomplished through management of the plants and other feed available in the diets of laying hens. In this study, the Ω -6/ Ω -3 fatty acid ratio, in addition to lutein content, was improved under a free-range production system in a rural area near Bogotá, Columbia. The fatty acid profile of plants available to the hens was analyzed to determine the source plants responsible for the increased yolk fatty acids via GC-FID. Two peaks appeared in plant extracts from native plants *Desmodium* sp. and *Sechium edule* which did not align with the retention time of available fatty acid standards, eluting between C16:0 and C17:0. Given that the extraction protocol and GC conditions were selected for fatty acid methyl esters, it was hypothesized that the unknown peaks were fatty acids, containing either a branch or double bond(s) (unsaturated). Samples were studied via a Waters SYNAPT G2 HDMS Q-TOF to identify candidate molecules for the two unknown peaks. Data was analyzed using Waters MassLynx and the Human Metabolome Database to identify possible compounds; the two unknown peaks were suggested to be a monounsaturated fatty acid and a polyunsaturated fatty acid. Peaks 1 and 2, with retention times of 14.55 and 15.68 min, had suggested empirical formulas of C₁₇H₂₈O₂ and C₁₈H₃₀O₂ and associated exact masses of 264.2089 and 254.2245 amu, respectively. With additional experiments, definitive peak identities will provide a more complete fatty acid profile of plants associated with the chicken's free range diet so that connections may be drawn between the plant's nutritional profile and the increased Ω -6/ Ω -3 fatty acid ratio found in chicken eggs bound for human consumers.

Poster Abstracts

#14 Developing a high throughput optomotor behavior assay in zebrafish larvae

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Photo-motor responses to exogenous chemicals in zebrafish larvae can provide insight into neurobehavioral changes caused by chemical and drug exposure. Zebrafish larvae are ideal for high-throughput behavior analysis due to their small size, color vision, and robust photo-motor responses. Ambient light levels, the presence of food, and zebrafish age influence their light/dark preferences. To assess zebrafish larval light/dark preference, an optomotor assay was developed using the Viewpoint Zebbox. The optomotor assay uses an LCD screen that can be programmed to vary the color and area of stationary light patterns. The LCD is immediately below the microtiter assay plate. Using 7- and 10-days post fertilization (dpf) larvae, the preference for white light compared to a dark environment (infrared light) was assessed. At this life stage, larvae preferred white light. An area of 25% white light, 75% dark, and one-minute area switching were optimal for the optomotor assay. Duration and sample size were assessed, and 5 minutes was determined to be sufficient to detect light/dark preference behavior in wildtype control larvae. To test the optomotor assay, larvae were exposed for 5 min to chemicals known to impact visual-motor behavior: 0.15% ethanol, 10 mg/L caffeine, and 0.05 mg/L benzodiazepine. Next steps include testing the optomotor behavior assay on younger larvae (5 dpf) as an effort toward higher throughput. A power analysis will be conducted to identify the minimum sample size needed to detect chemical impact to optomotor behavior. We believe that an automated approach to the optomotor assay will become a rapid tool to quickly uncover place preference abnormalities resulting from chemical exposure, a sensitive endpoint of larval zebrafish toxicology.

Poster Abstracts

#15 Improving an Antibiotic Resistance Database: Application of new visualization and Assembly Software

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The Functional Antibiotic Resistant Metagenomic Element (FARME) database is a compilation of publicly available DNA sequences and predicted protein sequences conferring antibiotic resistance (AR) as well as regulatory elements, mobile genetic elements and predicted proteins flanking AR genes. FARME DB focuses on functional metagenomic AR gene elements and provides a resource to better understand AR and the relationship between environmental AR sequences and AR genes derived from cultured isolates. FARME DB Website Sequence and Metadata consists of 20,724 FARME DNA sequences in FASTA format, 48,178 FARME predicted protein sequences in FASTA format 42,893 predicted hidden Markov models found within FARME DB protein sequences. FARME DB websites allows for interactive selection of individual project DNA sequence clones as well as using visualization of HMMs including AR, transcriptional regulators and mobile genetic elements. During this internship I was able to accomplish several key improvements in this AMR database in collaboration with the FRAME database team. First, QC measures identified and corrected outdated Javascript library links using PHP language website code.

(http://staff.washington.edu/jwallace/farme/chart_project_hmm.html). Second, a prototyped analysis of “MEGAHIT” Illumina paired-end read DNA contig assembly software using Docker and Amazon Web Services free tier Elastic Compute Cloud (EC2) was used for assembly improvement. Reanalysis of FAST5 format Oxford Nanopore long-read DNA signal files using “guppy” neural net software in “super” high accuracy mode (SUP) was conducted. I was able to install and test both GPU (graphics processing unit) and CPU only versions of Oxford Nanopore guppy software. These improvements demonstrated over two orders of magnitude improvement in processing speed using the GPU version of guppy. This allowed us to improve both public health and toxicology analysis of environmental antibiotic resistance.

Poster Abstracts

#16 Comparative Toxicity of Alkylated and Parent Polycyclic Aromatic Hydrocarbons in Primary Human Bronchial Epithelial Cells

Mackenzie Allison, Jamie Pennington, Brianna Rivera, Susan C. Tilton

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous contaminants released into the environment from multiple sources, including as byproducts of incomplete combustion of organic material resulting in formation of both substituted and unsubstituted (parent) PAH forms. Alkylated PAHs are substituted with hydrogen functional groups on the parent structure and are derived from crude oil and refined petroleum products. While these semi volatile PAHs are commonly found in both vapor and particle phases in air, little is known about their toxicity from inhalation exposure in the lung. In addition, because alkylated PAHs are not included in routine measurements and risk assessments, there is growing concern of underestimating overall PAH toxicity by ignoring this group of chemicals. Therefore, this study proposes to fill a significant data gap on toxicity of alkylated PAHs in primary human bronchial epithelial cells (HBEC) compared to parent PAHs. Both alkylated and parent forms of pyrene, phenanthrene and benzo[a]anthracene were tested in HBEC across dose-response for endpoints of cytotoxicity, oxidative stress, and mitochondrial membrane potential. Based on limited prior data in other test systems, we hypothesize that some alkylated PAHs will exhibit equivalent or higher toxicity compared to the parent form. Overall, the results showed that for the phenanthrene, pyrene and benz[a]anthracene groups, the alkylated product had equal or higher toxicity than the parent depending on the end point that was being tested. In addition, we observed that alkylated PAHs may cause toxicity through unique mechanisms compared to parent forms. These results indicate that current risk assessments may underestimate PAH risk from inhalation exposure due to lack of data for alkylated forms.

Poster Abstracts

#17 Size- and Shape Dependent Toxicity of Oxidation-Resistant Silver Nanoparticles

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Due to their unique physical and chemical properties, silver nanoparticles (AgNPs) are widely used in biomedical and commercial products. However, knowledge of AgNP toxicity is still limited and difficult to study due to the confounding presence of Ag⁺ ions released by oxidation of the particle surface. By coating the AgNPs with L-phosphatidylcholine (PC), sodium oleate (SOA), and hexanethiol (HT), it is possible to make oxidant-resistant AgNPs. Previous studies with oxidant-resistant AgNP spheres using a 5-day embryonic zebrafish assay showed size-dependent toxicity. The goal of this study was to observe differences in the toxicity of oxidant-resistant AgNPs of a variety of shapes and sizes using the same model assay. Dechorionated zebrafish embryo exposures began at 8 hours post fertilization (hpf). Toxicity was determined by observed zebrafish mortality and sublethal impacts assessed at 24- and 120-hpf. This study used AgNP spheres, triangles, and cubes; each particle shape came in sizes that varied between 20-100nm. Across all exposures that showed significant toxicity, a smaller particle size correlated with higher toxicity. The AgNP cubes showed no significant toxicity while the spheres and triangles showed distinct toxicity patterns with some unique malformations.

Poster Abstracts

#18 Assessing competitive metabolism of polycyclic aromatic hydrocarbons (PAHs) via in vitro metabolism

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Cytochrome P450s (CYPs) commence polycyclic aromatic hydrocarbon (PAH) metabolism, catalyzing chemical reactions to enhance solubility. However, bioactivation can occur, resulting in metabolites with increased toxicity. Human exposures commonly involve complex PAH mixtures, and multiple PAHs can act as substrates for various individual CYP enzymes. Our objective herein was to test the hypothesis that exposure to a PAH mixture can cause metabolic competition, leading to inhibition of PAH metabolism, which could then affect toxicity. We co-incubated binary mixtures of benzo[a]pyrene (BaP) and dibenzo[def,p]chrysene (DBC) in human hepatic microsomes and measured enzymatic loss of BaP and DBC. We determined inhibition coefficients (K_i) and observed competitive inhibition of both PAHs. We also measured K_i values of Supermix-10 (SM10), a mixture of the 10 most abundant PAHs found at the Portland Harbor Superfund Site, on BaP and DBC metabolism. SM10 inhibited both DBC and BaP metabolism. Of note, dose-dependent decreases seen in both BaP and DBC rates of metabolism suggests competition for the same enzymes. Overall, BaP inhibited DBC metabolism more potently than DBC inhibited BaP (0.061 vs 0.44 μM K_i , respectively). In addition, we developed a physiologically based pharmacokinetic (PBPK) interaction model by integrating PBPK models of DBC and BaP and incorporating measured metabolism inhibition coefficients. The PBPK model predicts significant increases in BaP and DBC concentrations in blood AUCs following high oral doses (≥ 100 mg), 5 orders of magnitude higher than typical exposures. Our results show these PAHs compete for the same enzymes and inhibit metabolism at high doses, but that BaP and DBC doses required for interaction are much higher than typical human exposure. Follow-up studies will investigate potential inhibition of SM10 metabolism by individual mixture components. Our research is helping to inform the human health risks posed by exposure to PAH mixtures.

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