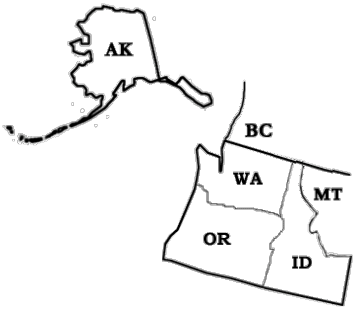

**Pacific Northwest Chapter, Society of Toxicology**

# Risk Assessment: From Hazard Identification to Risk Communication

PANWAT Regional Chapter Meeting

Seattle, Washington

September 20, 21, and 22, 2012

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# 2012 Regional Chapter Meeting

**September 20, 21, and 22, 2012**


**SHERATON SEATTLE HOTEL**
1400 Sixth Avenue, Seattle, WA 98101

**Risk Assessment: From Hazard Identification to Risk Communication**

Toxicologists assess safety of pharmaceuticals, agrochemicals, consumer products, industrial compounds, nanomaterials and other xenobiotics. Key roles of toxicologists are hazard identification, science-based risk assessment and clear risk communication. In support of this critical role, the Pacific Northwest Regional Chapter of Society of Toxicology will convene at the 2012 Annual PANWAT meeting and share recent advances in hazard identification and risk assessment of pharmaceuticals, pesticides, biotechnology products, nanomaterials, environmental contaminants and other xenobiotics.

## Detailed Program and Meeting Schedule

### Thursday, September 20th

4:30–8:00 pm **REGISTRATION**

6:30– 8:00 **Behavioral Interviewing – A key to hiring and getting hired**
Marlynn Haslund, MA, President and Founder of Ripple Effect Career Transition Coaching

8:00 **Networking – Elephant and Castle,**
1 block from Sheraton Hotel: 1415 5th Avenue Seattle, WA 98101

### Friday, September 21st

7:30 am-12:00 pm **REGISTRATION**

8:30--8:35 pm **Welcome remarks and conference overview**
PANWAT President: Dr. David Stone, Oregon State University

## SYMPOSIUM I: Hazard Identification and Risk Assessment of Pharmaceuticals and Biotech Products

8:35-9:00 am **Continuous Cardiovascular Safety Assessment in Repeat-Dose Studies**
Robert Kaiser, PhD, DABT, Senior Research Scientist at Charles River Laboratories

9:00-9:25 **Juvenile Toxicity Testing to Support Clinical Trials in the Pediatric Population**
Ali Faqi, PhD, Senior Director Developmental & Reproductive Toxicology at MPI Research

9:25-9:50 **Conduct of Developmental and Reproductive Toxicology Studies in Nonhuman Primates: An Update**
Norbert Makori, PhD, Director, Developmental and Reproductive Toxicology at SNBL

9:50-10:05 **AM Coffee Break and Networking**

10:05-10:25 **Emixustat (ACU-4429): Comparative Preclinical Ocular Distribution in Rat, Dog, and Monkey to Support Clinical Risk Assessment**
Terry Podoll, PhD, Senior Scientist at Acucela

10:25-10:50 **CBRN Countermeasure Approval under the Animal Rule**
Jay S Charleston, PhD, Sr. Program Manager, Preclinical, Sarepta Therapeutics

10:50-11:15 **Interference With Bile Acid Excretion is a Susceptibility Factor for Clinical Hepatotoxicity**
Ryan E. Morgan, MS, DABT, Scientist, Toxicologist at Amgen

11:15-11:35 **New Approaches for the Development of Genomic Biomarkers in Toxicology**
Mark Parrish, Associate Scientific Director of Biomarker Translation and Assay Development, Covance Genomics Laboratory

11:35 am-1:00 pm **Lunch Break on own – Seattle - Networking**

1:00-1:05 pm **Reconvene Conference**
David Stone, PhD, President of PANWAT

## KEYNOTE ADDRESS

1:05-1:45 pm ***Keynote Speaker:*** **Winner of PANWAT Achievement Award!**
Robert Tanguay, PhD, Oregon State University


## SYMPOSIUM II: Human Health Risk Assessment

1:45-2:10 pm **The Strategy Used to Build a Toxicological Database for Emerging Chemicals Focusing on the Mechanism of Action**
Richard C. Pleus, PhD, Managing Director of Intertox, Inc.

2:10-2:35 **Case Studies in Risk Assessment and Risk Communication**
Thomas A. Lewandowski, Ph.D., DABT, ERT, Toxicologist at Gradient

2:35-3:00 **Risk Assessment of Dietary Supplements and Functional Foods**
Alexander G. Schauss, PhD, FACN, Senior Director of Research and CEO, AIBMR Life Sciences

3:00-3:15 **PM Coffee Break and Networking**

3:15-3:40 **A Dimethyl Mercury Inhalation Risk Screening Concentration for Public Health Protection**
Matt Kadlec, PhD, DABT, Senior Toxicologist at Washington Department of Ecology Air Quality Program

3:40-4:00 **A Systems-based Multimodal Imaging and Computational Modeling Approach for Assessing Neurotoxicity**
Charles Timchalk, PhD, Scientist and Toxicologist at Systems Toxicology, Pacific Northwest National Laboratory

4:00-4:15 **BREAK and POSTER SET-UP**

4:15 to 6:00 **RECEPTION AND POSTER PRESENTATION**
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**Saturday, September 22**

## SYMPOSIUM III: RECENT ADVANCES IN TOXICOLOGY

8:30-8:40 pm **Reconvene Conference**
Dr. David Stone, President of PANWAT

8:40-9:00 **Neurotoxicity in developing zebrafish embryos caused by ligand-induced G-protein coupled receptor (GPER) inhibition.**
Siba R. Das, Dept of Environmental and Molecular Toxicology, Oregon State University.

9:00-9:20 **Defining the Developmental Toxicity of Environmentally relevant Oxygenated Polycyclic Aromatic Hydrocarbons (OPAHS) Using Embryonic Zebrafish**
Andrea L. Knecht, Dept. Environmental and Molecular Toxicology, and the Environmental Health Sciences Center, Oregon State University

9:20-9:40 am **Global Gene Expression Analysis Reveals Putative Signaling Pathways Underlying Neurobehavioral Toxicity of Bisphenol A Exposure in Embryonic Zebrafish**
Katerine S. Saili, Dept. of Environmental and Molecular Toxicology, Environmental Health Sciences Center, Oregon State University

10:00-10:10 **AM Coffee Break**

10:10-10:30 **Zebrafish Xenograft Model of Glioblastoma to Investigate Structure Activity Relationships of Zinc Oxide Nanoparticles with Anticancer Properties**
Leah C. Wehmas, Dept. of Environmental and Molecular Toxicology, The Sinnhuber Aquatic Research Laboratory and the Environmental Health Sciences Center, Oregon State University, Corvallis, Oregon, USA; [2] The Oregon Nanoscience and Microtechnologies Institute and the Safer Nanomaterials and Nanomanufacturing Initiative.

10:30-10:50 **Anti Androgen Flutamide Targets The Aryl Hydrocarbon Receptor to Inhibit Cancer Cell Proliferation**
Daniel Koch, Cancer Research Laboratory and Dept. of Environmental and Molecular Toxicology, Oregon State University, Oregon State University

10:50-11:10 **The Role of IL-2 Signaling in Aryl Hydrocarbon Receptor (AhR)-Mediated Immunosuppression**
Diana Rohlman, Environmental and Molecular Toxicology, Oregon State University, Corvallis OR, 97333

11:10-11:20 **PANWAT and Student Poster Awards**
Dave Stone, PhD, President of PANWAT

11:20 am **ADJOURN**
Dave Stone, PhD, President of PANWAT

# Presentation Abstracts

## Thursday, September 20th

**6:30-8:00 pm**

### Behavioral Interviewing – A key to hiring and getting hired

**Marlynn Haslund, MA, President and Founder of Ripple Effect Career Transition Coaching**

Behavioral interviewing is the most effective way to find the right job candidate. Behavioral Interviewing (BI) is the technique of using open ended questions and following-up with specific clarifying questions to determine if the candidate really has demonstrated the needed skill or behavior in the past. The use of BI increases successful hiring as measured by lower turnover, lower legal issues, and increased employee satisfaction. For success, the interviewee needs to respond to questions with a descriptive answer that explains the **S**ituation, **A**ction taken and **R**esult. I nterviewers will learn and practice how to ask the right questions. Interviewees will learn how to prepare and answer key questions in a Behavioral Interview. Learn how to cut through the predictable interviewing questions and focus on the behaviors needed to succeed on the job.

## Friday, September 21st

## SYMPOSIUM I: Hazard Identification and Risk Assessment of pharmaceuticals and biotech products

**8:35-9:00 am**

### Continuous Cardiovascular Safety Assessment in Repeat-Dose Studies

**Robert Kaiser, PhD, DABT, Senior Research Scientist at Charles River Laboratories**

Innovations in safety pharmacology technology coupled with enlightened approaches to assessing risk and meeting regulatory requirements for drug development have created opportunities to conduct multi-purpose studies in single cohorts of animals. When properly conducted, this creates efficiencies in time, test material, resources, and animal use as well as increasing the power of the safety assessment. Cardiovascular assessment is a perfect candidate for such study hybridization, as educated incorporation of ECG and/or blood pressure evaluation into repeat-dose studies affords the efficiencies defined above as well as generates valuable chronic exposure information not captured in stereotypic single-dose safety pharmacology study designs. This approach is provided for in regulatory documents and applicable in increasing numbers of therapeutic platforms and indications. We have evaluated both internal and external telemetry technology for utility in repeat-dose studies to generate continuous requisite data for cardiovascular safety assessment. Further, we have catered approaches to interpretation and analysis of these data in the toxicology study setting. These technologies have proven efficient and powerful for detecting changes in blood pressure (±6 mmHg) and QT interval (6-10 ms) in the repeat-dose setting, but must be implemented properly to avoid confounding either (or both) toxicology and safety pharmacology datasets.

**9:00-9:25 am**

## Juvenile Toxicity Testing to Support Clinical Trials in the Pediatric Population

**Ali Faqi, PhD, Senior Director Developmental & Reproductive Toxicology at MPI Research**

Substantial anatomical, biochemical, and physiological changes occur during infancy, childhood, and adolescence. These maturational changes can substantially affect the absorption, distribution, metabolism, and elimination of xenobiotics.

Many therapeutic drugs used in children have only been tested in adults and therefore, lack adequate information in the labeling for use in pediatrics. Inadequate pediatric labeling may expose children to the risk of unexpected adverse reactions or lack of optimal treatment. Therefore, the development of additional information in pediatric patients is required to provide appropriate dosing recommendations as correct pediatric dosing cannot necessarily be extrapolated from adult dosing information. The purpose of this presentation is to discuss the regulatory requirements of pre-clinical juvenile animal testing; case-by-case based study designs, species selections, relevant endpoints, and logistical and technical limitations as well as the application of juvenile toxicity data in the risk management in human.

**9:25-9:50 am**

## Conduct of Developmental and Reproductive Toxicology Studies in Nonhuman Primates: An Update

**Norbert Makori, PhD, Director, Developmental and Reproductive Toxicology at SNBL**

The nonhuman primate (NHP) in recent years has become a common preclinical *in vivo* model for drug development as one of the main predictors of reproductive and developmental toxicity outcomes. Main study designs encompass test compound administration to sexually mature NHPs in repeat dose toxicology and pregnant NHPs in developmental toxicity. This presentation provides an overview of conduct of these types of studies in relation to the ICH S6 guideline and practical industry experience. Examples of challenges in data interpretation include high early pregnancy losses and unexpected outcomes during or shortly after delivery of newborns. Evaluation of the immune system postnatal in NHP infants has routinely been confined to lymphocyte immunophenotyping, TDAR and histology. However, technical challenges for *in vitro* assays including NK and CTL assessments are being overcome, and will be discussed.

**10:05-10:25 am**

## Emixustat (ACU-4429): Comparative Preclinical Ocular Distribution in Rat, Dog, and Monkey to Support Clinical Risk Assessment

**Terry Podoll[1], Russell Eyre[1], Eric Austin[1], Elizabeth Prescott[2], Gang Sun[2], Suliman Al-Fayoumi[1], Mark Orme[1], and Ryo Kubota[1].** *[1]Acucela Inc., Seattle, WA; [2]Covance Laboratories Inc., Madison, WI*

Emixustat hydrochloride (ACU-4429) is a visual cycle modulator that inhibits the retinal isomerase enzyme, RPE65, responsible for a key step in the regeneration of 11-*cis*-retinal. Emixustat is currently in Phase 2 clinical development as an orally administered treatment for

dry AMD. Other than ocular effects, no systemic toxicity was observed at dose levels up to 30 mg/kg/day in the rat and up to 60 mg/kg/day in the dog and monkey. In ADME studies, systemic exposures to emixustat were a small fraction of the cumulative exposure to total radioactivity due to extensive metabolism producing pharmacologically inactive metabolites. To support the risk assessment of emixustat clinical exposure, ocular distribution studies using $^{14}$C-emixustat were undertaken in rat, dog, and monkey. Consistent among the three species, emixustat parent molecule was the major component found in relevant eye tissues. In all three species, peak concentrations ($C_{max}$) of emixustat in the retina were >50-fold higher than respective peak plasma levels on a ng•equiv/g basis. Although high levels of the major metabolites were observed in plasma, they were not observed in eye tissues. Preclinical ocular distribution results indicated emixustat parent molecule is the predominant component responsible for clinical outcomes.

**10:25-11:50 am**

## CBRN Countermeasure Approval under the Animal Rule

**Jay S Charleston, PhD, Sr. Program Manager, Preclinical, Sarepta Therapeutics**

Exposure to Chemical, Biological, Radiological and Nuclear (CBRN) hazards represent potential for permanent disabilities or death. These CBRN agents represent natural, military, and terrorism threats. The development of Medical Counter Measures for these threats is not practical or ethical under normal drug development pathways since exposure to humans in clinical trials cannot normally be used to demonstrate efficacy. The Animal Rule provides a development pathway wherein surrogate models of the CBRN toxic agent effects may be used to infer efficacy in human populations. An overview of the Animal Rule Essentials and other requirements and expectations will be discussed when using the Animal Rule to support the development of Medical Counter Measures.

**11:50-11:15 am**

## Interference with Bile Acid Excretion is a Susceptibility Factor for Clinical Hepatotoxicity

**Ryan E. Morgan, MS, DABT.** *Department of Comparative Biology and Safety Sciences, Discovery Toxicology, Amgen, Thousand Oaks, CA 91320, USA***.**

A significant challenge in drug development is that traditional preclinical animal models, such as rodents or even non-human primates, sometimes fail to predict liver injury in humans. An example is given with Compound X, a proprietary small molecule that was being developed for an inflammatory disorder. This compound showed no evidence of liver injury in multiple preclinical models, and at dose levels and exposures well above what was intended for humans. In a phase 1 human trial, in healthy volunteers, 5 of 8 patients showed significant liver enzyme elevations that resulted in cessation of the trial. Subsequent *in vitro* studies were conducted to evaluate reactive metabolite formation, covalent binding, mitochondria toxicity, and other cytotoxicity endpoints. Compound X was negative in all *in vitro* studies up to precipitating concentrations. Gene expression analysis was performed on livers from rats treated with Compound X for 14 days (qd dosing). Microscopic evaluation of these animals confirmed that the livers were normal, with no evidence of injury. Using stringent statistical

cutoffs, 9 genes changed in a dose dependent and statistically significant manner. Based on literature and pathway interrogations, these genes appeared to play a role in bile acid and cholesterol homeostasis. Further review of the literature implicated bile salt export pump (BSEP) inhibition as a possible cause for liver injury in humans that may go unpredicted by rodents. Additional *in vitro* studies were conducted on Compound X, and BSEP inhibition was confirmed. Inhibition of rat BSEP and human BSEP was represented by an $IC_{50}$ value of ~ 11µM for both species. To better understand the association between BSEP interference and liver injury in humans, > 600 benchmark compounds (mostly comprised of marketed or withdrawn drugs) were evaluated in a membrane vesicle system. Most compounds with an $IC_{50}$ potency of ≤ 25µM in the BSEP vesicle transport assay have convincing evidence of liver injury in humans, indicating that BSEP interference is likely a susceptibility factor. However, some benchmarks with potent BSEP $IC_{50}$ values show little or no evidence of liver injury in humans, so additional transporters involved in bile acid transport were evaluated in an attempt to further discriminate compounds. The effect of all 600+ benchmark compounds referenced above on multidrug resistance-associated proteins (MRP) 2, 3, and 4 were evaluated in a membrane vesicle assay. Hierarchical cluster analysis of BSEP, MRP2, MRP3, and MRP4 $IC_{50}$ values is presented, along with details about how the compounds clustered, and how these data might best be integrated into a multi-factorial approach to drug safety assessment. We conclude that although BSEP interference, alone, appears to be a strong predictor of liver injury in humans, integration of BSEP data with that of other transporters, other *in vitro* endpoints, measured or predicted exposure in humans, and possibly other parameters are needed to best resolve drugs with a high risk of liver injury in humans from those with little or no risk.

**11:15-11:35 am**

## New Approaches for the Development of Genomic Biomarkers in Toxicology

**Mark Parrish, Associate Scientific Director of Biomarker Translation and Assay Development, Covance Genomics Laboratory**

Genomics tools are often used as a means of identifying biomarkers of disease and therapeutic response. These same platforms can also be leveraged for the development of toxicogenomic biomarkers, as well as to elucidate underlying biological mechanisms. This presentation will highlight some interesting examples of how gene expression and miRNA profiling are being utilized in toxicology studies.

## Friday, September 21st

# KEYNOTE ADDRESS

**1:05-1:45 pm**

***Keynote Speaker:*** **Winner of PANWAT Achievement Award!**

**Robert Tanguay, PhD, Oregon State University**

## SYMPOSIUM II: Human Health Risk Assessment

**1:45-2:10 am**

### The Strategy Used to Build a Toxicological Database for Emerging Chemicals Focusing on the Mechanism of Action

**Richard C. Pleus, PhD, Managing Director of Intertox, Inc.**

The scientific literature still does not adequately understand potential risks to human health and the environment posed by many chemicals currently on the market (e.g. ingredients in pharmaceuticals or personal care products), and more emerging chemicals of concern (ECCs) enter the market each year. It thus falls on toxicologists to perform cost-effective and scientifically valid research as quickly as possible, so as to determine how to best protect the public without causing unnecessary hardship to the private sector. The traditional toxicological approach to understanding the potential risk of a chemical involves a progression of animal assays to determine a relationship between dose and effect. This approach is costly, time-consuming, and approximate. Toxicologists are developing methods to more precisely determine chemical cause and effect, chief among them determination of the Mechanism of Action (MoA), the biochemical pathway through which a particular chemical causes a particular effect. This talk discusses the strategy used to determine a conservative point of departure for perchlorate, a chemical that is found in many products, most notably in rocket fuel. Researchers performed the following:

1. Aggregated known data into a toxicological database;
2. Determined the most sensitive endpoints;
3. Assessed what was scientifically reliable;
4. Add new research.

It was found that perchlorate's MoA was iodide uptake inhibition (IUI) in the thyroid, and that at or below a dose of .007 mg/kg per day perchlorate does not cause IUI.

**2:10-2:35 pm**

## Case Studies in Risk Assessment and Risk Communication

**Thomas A. Lewandowski, Ph.D., DABT, ERT, Toxicologist at Gradient**

Risk assessment provides the bridge between toxicology research and public health policy. While the toxicologist's role can be limited to that of data generator, it is increasingly common for toxicologists to be involved in the broader aspects of risk assessment and to participate in discussions in areas beyond traditional toxicology. Three case examples will be used to illustrate these points. The first involves a risk assessment to determine the acceptability of new chemical refrigerants. The current class of refrigerants are being eliminated due to their global warming properties but the selection of alternatives has been no easy matter with the need to consider trade-offs in environmental impacts and chemical safety. In this context, risk assessment provides a way of balancing different types of risks and holding them within acceptable limits. The second case study involves a risk assessment of nanosilver coatings on medical devices. The challenges here involve a limited toxicology dataset and the rapidly expanding use of nanomaterials in consumer products. The final case example involves a study of the possible association between mercury exposure and autism. Focused more on epidemiology than toxicology, the lessons to be learned here include the limited ability of research to inform public opinion in cases where science is already highly politicized and emotionally loaded.

**2:35-3:00 pm**

## Risk Assessment of Dietary Supplements and Functional Foods

**Alexander G. Schauss, PhD, FACN, Senior Director of Research and CEO, AIBMR Life Sciences**

Safety assessment of dietary supplements and functional foods can present a significant challenge to toxicologists. Unlike single molecules or biologics, supplements and foods can contain thousands of compounds, many of which are bioactive. The importance of determining the safety of all supplements and foods can be illustrated by the discovery of a delicious fruit found in the tropics, and proposed as a juice for chronic consumption, that was found to cause atypical Parkinson’s disease, despite initial skepticism that it had a “history of traditional use.” Toxicological studies have become essential in preparing submissions of new dietary ingredients intended to be sold as dietary supplements, as well as ingredients seeking generally recognized as safe (GRAS) affirmation status for their intended use in foods. There exists a need to consider drug-nutrient and drug-phytochemical interactions given the prevalence of consumers taking prescription and OTC drugs. Adverse event databases are proving useful in determining to what degree concerns with the safety of these products is warranted.

**3:15-3:40 pm**

## A Dimethyl Mercury Inhalation Risk Screening Concentration for Public Health Protection

**Matt Kadlec, PhD, DABT, Senior Toxicologist, Washington Department of Ecology Air Quality Program**

Dimethyl mercury (DMM), CASRN 593-74-8, is a small component of headspace gas emissions from some waste storage tanks near the Hanford nuclear waste treatment plant. DMM vapor is also emitted by municipal landfills. It is a trace gas in the global mercury cycle and is sometimes detected in fish tissue along with monomethyl mercury ($CH3Hg^{+}$). Acute DMM exposures of as little as 5-mg/Kg body weight have caused delayed brain damage and death to humans. Fetal neurological development is likely the effect induced by the lowest exposures. As with monomethyl mercury, this might occur from very low maternal exposures to DMM. Occupational exposure limits for alkyl mercury compounds have been published; however, inhalation limits, such as an MRL or RfC, for public exposure to any alkyl mercury compounds are unavailable. The literature suggests DMM is biologically inactive until it undergoes demethylation. This presentation shows how I derived a risk screening concentration for DMM of 0.14-µg/$m^3$ (daily time-weighted average) using published data and assumptions about absorption, distribution, metabolism and elimination kinetics. Public exposure outside the Hanford area boundary from inhalation and ingestion of DMM emissions from the waste tank transfer ventilation systems is 6.9E-07-µg/Kg body weight per day or less. These emissions appear to pose no appreciable off-site health risks.

**3:40-4:00 pm**

## A Systems-based Multimodal Imaging and Computational Modeling Approach for Assessing Neurotoxicity

**Charles Timchalk and Kevin Minard, Pacific Northwest National Laboratory, Richland, WA**

Recent epidemiology studies suggest a strong association between low-level exposures to environmental chemicals and impaired child neurodevelopment. To elucidate possible mechanisms, we describe a systems-based toxicology approach that exploits a computational framework to integrate biological response data and identify underlying perturbations to cellular and system response pathways. The basic strategy employs the latest developments in multimodal imaging to capture adverse biological response across scales that span from the cell and organ to the living organism. New bioinformatic tools are then applied to rapidly assess adverse toxicological outcomes resulting from the perturbation of specific pathways critical for normal cell and organ function. To illustrate possible performance and limitations, we describe a case study of neurotoxicity that is readily induced and manipulated in rodents with Kainic Acid (KA). We discuss how biomedical imaging and spectroscopy can be exploited to evaluate the kinetics of KA response *in vivo,* and how measured outcomes monitor key Mode-of-Action (MOA) endpoints that include ionic imbalance, neuronal swelling, altered metabolism, neuron-astrocyte coupling, changes in neurotransmitters levels, mitochondrial dysfunction, cell death, tissue edema, glial scarring, breakdown of the blood brain barrier, and altered blood flow. We also explore how recent advances in imaging mass spectrometry (IMS) can be exploited to comprehensively evaluate underlying omic-level changes in the context of

localized target tissue cellular dose. We further illustrate how these diverse data types are compiled within a computational brain atlas that automatically extracts multimodal imaging results for integrated statistical and bioinformatics that identify and characterize the dose-response of toxicity pathways for different anatomical brain regions. It is envisioned that this multi-modal imaging and systems toxicology paradigm is capable of integrating dosimetry and biological response across spatial and temporal domains to streamline current risk-based toxicity testing.

4:15 to 6:00 pm **RECEPTION AND POSTER PRESENTATION**

POSTER ABSTRACTS ARE AT END OF THIS DOCUMENT.

## Saturday, September 22

## SYMPOSIUM III: RECENT ADVANCES IN TOXICOLOGY

**8:40-9:00 am**

### Neurotoxicity in Developing Zebrafish Embryos Caused by Ligand-Induced G-Protein Coupled Receptor (GPER) Inhibition.

**Siba R. Das and Robert L. Tanguay.** *Dept of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR*

G protein-coupled estrogen receptor 1 (GPER) is a G protein-coupled receptor (GPCR) unrelated to nuclear estrogen receptors but strongly activated by 17β-estradiol in both mammals and fish. In zebrafish GPER is expressed as early as 1 hr post fertilization (hpf) and exhibits strong stage-dependent expression patterns during embryogenesis. At 26 and 38 hpf, GPER mRNA is broadly distributed throughout the body, whereas from 50 to 98 hpf, it is increasingly localized to the heart, brain, neuromasts, craniofacial region, and somite boundaries of developing zebrafish. It has been documented that exposure to the agonist of GPER (G1) causes malformations in developing zebrafish embryos, but no known adverse effects has been associated with exposure to the antagonist, G15. The present study was aimed at delineating toxicity observed in zebrafish larvae due to ligand-induced inhibition of GPER. Results indicated that continuous exposure to a selective GPER antagonist, G-15 from 8 to 120 hpf, or within three developmental windows ranging from 8 to 72 hpf, resulted in adverse concentration-dependent effects on their responsiveness to touch. The larvae exposed to G15 also, show a significant reduction in their movement in an assay using absence of light as a stimulus. Zebrafish larvae that were co-exposed to both G15 and G1 together didn't have the same lack of responsiveness to touch suggesting that G-15 toxicity is mediated via aberrant inhibition of GPER. These studies were sponsored by NIH P30ES00210 and 1R21ES018970.

**9:00-9:20 am**

## Defining the Developmental Toxicity of Environmentally relevant Oxygenated Polycyclic Aromatic Hydrocarbons (OPAHS) Using Embryonic Zebrafish

**Andrea L. Knecht[1], Britton Goodale[1], Annika Swanson, Michael Simonich, and Robert L.Tanguay[1].** *[1]Department of Environmental and Molecular Toxicology, and the Environmental Health Sciences Center, Oregon State University, Corvallis, OR*

Oxygenated polycyclic aromatic hydrocarbons (OPAHs) are produced by incomplete combustion and oxidation of parent PAHs. OPAHs are widely present in the environment and may pose a risk to human health, making their timely evaluation important. We used a rapid throughput zebrafish developmental screen to evaluate the toxicity of a structurally diverse set of OPAHs. Dechorionated embryos were exposed between 6 and 120 hours post fertilization (hpf) and evaluated for mortality and a suite of complex endpoints at 120hpf. In addition, IHC was conducted to evaluate AHR activation through the induction of the downstream target, CYP1A. Representative subsets of OPAHs were selected for further analysis based on differential toxicity and AHR-dependent CYP1A expression profiles. The Seahorse Extracellular Flux Analyzer was used to measure *in vivo* oxidative stress in 24hpf zebrafish embryos and qRT-PCR was conducted at 48hpf using primers for a number of DNA repair and oxidative stress genes important in cellular detoxification and protection from oxidative damage. These data represents an *in vivo* toxicity characterization of environmentally relevant OPAHs revealing a structure toxicity relationship, differential AHR dependencies, and a prominent role for oxidative stress in the toxicity mechanisms. This research was supported by the NIEHS grant P42ES016465 and P30ES00210.

**9:20-9:40 am**

## Global Gene Expression Analysis Reveals Putative Signaling Pathways Underlying Neurobehavioral Toxicity of Bisphenol A Exposure in Embryonic Zebrafish

**Katerine S. Saili*, Margaret M. Corvi*, Siba R. Das*, Susan C. Tilton[†], Katrina M. Waters[†], and Robert L. Tanguay*.** ***Department of Environmental and Molecular Toxicology, Environmental Health Sciences Center, Oregon State University, Corvallis, OR; [†]Computational Biology and Bioinformatics Group, Pacific Northwest National Laboratory, Richland, WA*

Bisphenol A (BPA) is an endocrine disrupting compound widely used in consumer product manufacturing. Transient 0.1 µM BPA exposure results in larval zebrafish hyperactivity and adult learning impairments. The mode of action underlying these effects presumably involves impacts on nervous system development through activation of classical estrogen receptors (ERs) or other BPA receptors such as estrogen related receptor gamma (ERR3). We used global gene expression analysis to investigate the transcriptional level effects of BPA exposure and to identify candidate genes and signaling pathways that mediate BPA's developmental toxicity in zebrafish. Embryos were exposed from 8 to 24 hours post fertilization to 0.1% DMSO, 0.1 µM BPA, 0.1 µM 17β-estradiol, or 0.1 µM GSK4716 (an ERR3 agonist). Processed total RNA samples were hybridized to a 135K NimbleGen microarray. Functional analysis of differentially expressed genes revealed prothrombin activation as a top canonical pathway impacted by both BPA and E2 exposure, and suppressed expression of key genes involved in

nervous system development and function, putatively through ERR3 activation, following BPA and GSK4716 exposure. These results provide insight into potential modes of action underlying BPA's neurodevelopmental toxicity in zebrafish.

Supported by NIEHS T32ES7060, 1R21ES018970, P30 ES000210, and an EPA STAR Graduate Fellowship to KSS.

**10:10-10:30 am**

## Zebrafish Xenograft Model of Glioblastoma to Investigate Structure Activity Relationships of Zinc Oxide Nanoparticles with Anticancer Properties

**Leah C. Wehmas[1,2], Lisa Truong[1,2], Jeffrey A. Greenwood[3], Alex Punnoose[4], & Robert L. Tanguay[1,2].** *[1] Department of Environmental and Molecular Toxicology, The Sinnhuber Aquatic Research Laboratory and the Environmental Health Sciences Center, Oregon State University, Corvallis, Oregon, USA; [2] The Oregon Nanoscience and Microtechnologies Institute and the Safer Nanomaterials and Nanomanufacturing Initiative; [3] Department of Biochemistry and Biophysics, Oregon State University, Corvallis, Oregon, USA; [4] Department of Physics, Boise State University, Boise, Idaho, USA*

Zinc oxide nanoparticles (ZnO-NPs) demonstrate preferential cytotoxicity toward cancer cells in culture. We developed a NP screening paradigm by xenotransplanting human glioblastoma cells into the cranium of zebrafish to assess the physicochemical properties of ZnO-NPs that enhance cytotoxicity to cancer cells. The goal is to identify ZnO-NPs that inhibit cancer cell proliferation. We investigated the role of charge, size, Fe as a ROS catalyst and route of exposure (waterborne vs. micro-injection) on the toxicity of ZnO-NPs in embryonic zebrafish. Generally, the ZnO-NPs did not induce significant adverse effects below 10 $\mu g \cdot ml^{-1}$. Charge influenced the biological response with highly positive NPs inducing less response than nearly neutral NPs, depending on route of exposure. Reducing the ZnO-NP size was associated with more malformations. Increasing the Fe content amongst similarly sized and positively charged ZnO-NPs induced greater adverse response in zebrafish via waterborne exposure. An optimized ZnO-NP was synthesized (mean diameter=8.35 nm; zeta potential=+43.6 mV) and preliminary results demonstrate inhibition of glioblastoma cell proliferation at 0.1 mM. Our screening paradigm holds promise for identifying physicochemical traits which enhance the anti-cancer properties of ZnO-NPs while supporting safe NP design.

Research support: NSF 134468, NIEHS P30ES000210, ES016896, T32ES07060, and Air Force Research Laboratory #FA8650-05-1-5041.

**10:30-10:50 am**

## Anti Androgen Flutamide Targets The Aryl Hydrocarbon Receptor to Inhibit Cancer Cell Proliferation

**Daniel Koch [1,2], Edmond O'Donnell[1,2], Hyo Sang Jang[1], William Bisson[1], Prasad Kopparapu[1,2], Nancy Kerkvliet[1], and Siva Kumar Kolluri[1,2].** *[1]Cancer Research Laboratory, Oregon State University, Corvallis OR 97333; [2]Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis OR 97333*

The aryl hydrocarbon receptor (AhR) is a member of the PER/ARNT/Sim (PAS) domain family of transcription factors responsive to exogenous signals, and is recognized as the mediator of dioxin toxicity. The AhR also exerts influence on many cellular processes relating to cellular growth and differentiation in a ligand-dependent manner. We postulated that AhR ligands could be identified that repress cancer cell growth. In order to identify such AhR ligands, we conducted a small molecule screen and discovered flutamide as one of the putative ligands. Flutamide is used clinically as an androgen receptor antagonist for the treatment of prostate cancer. We demonstrate that flutamide causes cytosol to nuclear translocation of the AhR and stimulates the transcriptional activity of the AhR. We also identify the AhR as a mediator of flutamide-induced anti-proliferative effects in cancer cells. Furthermore, we identify the downstream mediators of flutamide-induced, AhR-dependent growth inhibition of cancer cells.

**10:50-11:10 am**

## The role of IL-2 signaling in aryl hydrocarbon receptor (AhR)-mediated immunosuppression

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The AhR, activated by the immunosuppressive environmental contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), induces $Foxp3^{+}$ and $Foxp3^{neg}$ T-regulatory cells (Treg). Tregs negatively regulate T-cell responses, including the cytotoxic T-lymphocyte (CTL) response in graft-versus-host disease (GVHD). In GVHD TCDD induces a $CD4^{+}CD25^{+}Foxp3^{neg}IL\text{-}10^{+}$ Treg phenotype that precedes suppression of the CTL response. The high affinity receptor for IL-2, CD25 is associated with regulatory function, while IL-2 is required for Treg differentiation. The IL-2 gene contains three dioxin response elements, suggesting TCDD may induce IL-2, thus driving the $CD25^{+}$ phenotype. The purpose of this study was two-fold: 1) to determine if excess IL-2 drives the Treg phenotype in the absence of TCDD and 2) to determine if CD25 expression is required for TCDD-mediated immunosuppression. Animals were independently dosed with either exogenous IL-2 or an anti-CD25 antibody to block CD25 expression. While excess IL-2 partially recapitulated the Treg phenotype and suppressed the CTL response, blockade of CD25 was insufficient to rescue TCDD-mediated immunosuppression. Unexpectedly, treatment with TCDD increased Foxp3 expression on donor T-cells on day 15. However, suppression of Foxp3 by anti-CD25 treatment did not alter suppression of the CTL response by TCDD. The apparent lack of involvement of CD25 or Foxp3 indicates TCDD may induce Tr1 cells, characterized by expression of IL-10.

# Poster Abstracts

### Inductive Effects on Reactivity of the Contact Allergen Benzoquinone to Proteins

**W Mbiya[1,*], I Chipinda[2], P. D. Siegel[2] and R. H. Simoyi[1].** *[1]Department of Chemistry, Portland State University, Portland, OR 97207-0751. [2]Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown,WV 26505-2888*

Benzoquinone (BQ) and substituted benzoquinones (SB) are used for dye and cosmetics production and also as precursors. BQ is an electrophile known to covalently modify proteins via Michael Addition (MA) but the reactivity, reaction mechanistic domains and allergenicity of SB are unknown. Electron withdrawing and electron donating substituents on BQ were assessed for effects on BQ reactivity and allergenicity. Alternative potential protein binding mechanisms were explored. BQ binding to Cys34 on human serum albumin (HSA) was studied and for BQ and SB reactivity studies, nitrobenzenethiol (NBT) was used as a surrogate for protein. Hammett and Taft (HT) constants were also used to evaluate the influence of these substituents on chemical reactivity. Both NBT binding studies and HT values demonstrated chlorine SB to be more reactive than methyl and t-butyl SB. Production of semiquinone radicals and characterization of SB-NBT adducts demonstrate that haptenation may also occur via free radical and vinylic substitution mechanisms, in addition to MA. BQ and SB dermal allergenicity in mice was consistent with that predicted by reactivity and HT data. These results demonstrate the effect of substituents on BQ reactivity and allergenicity whilst suggesting potential utility of chemical reactivity data for electrophilic allergen identification and potency ranking.

This work was supported by the NIOSH/NIEHS IAG#Y1-ES-0001-12 and NSF CHE 105631.

### Shipping of Non-Clinical Flow Cytometry Specimens: The Ideal Condition for Consistency of Sample Handling Throughout the Drug Development Process

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**Rationale:** A wide variety of specimen types, including whole blood, peripheral blood mononuclear cells (PBMC) and various tissues can be analyzed by flow cytometry for drug development studies. When shipping samples to a laboratory for analysis, cell integrity, morphology and antigenic site activity can be compromised due to unfavorable environmental conditions and delivery delays. However, shipping of samples has many benefits such as an increase in choice of testing laboratories, improved consistency using the same laboratory for non-clinical as well as clinical analysis and maximizing the use of laboratories that specialize in specific types of testing, such as flow cytometry. **Experimental Procedures:** In our study we tested non-human primate (NHP) whole blood specimens shipped overnight in Cyto-Chex® BCT (blood collection tubes) and compared the results to more traditional anticoagulants such as Na Heparin and EDTA. Whole blood immunophenotyping of CD3, CD4, CD8, CD159a, and CD20 on NHP blood was evaluated by flow cytometry analysis on a BD FACSCanto™ equipped with BD FACSDiva™ software. Rodent tissue specimens were shipped in 100% FBS and the resulting cell suspensions were evaluated for cell count and viability on a Guava® PCA equipped with Cytosoft™ software. **Results:** Our study showed that NHP whole blood specimens can keep their integrity for 24-48 hours using EDTA tubes,

72 hours using Na Heparin tubes and up to one week using Cyto-Chex® BCT when held at room temperature. For the tissue specimens, viability and cell count can be directly impacted by shipping temperature. Shipping tissue specimens on frozen gel packs, as opposed to refrigerated gel packs, caused a decrease in viability from 82.2 – 91.1% (frozen gel packs) vs. 50.7 - 72.2% (refrigerated gel packs). **Conclusion:** Despite the risks of shipping, monitoring shipping temperature and use of a stabilizing anticoagulant that helps maintain whole blood sample integrity for longer periods can make shipment of non-clinical samples more reliable and allow for greater flexibility in the choice of flow cytometry laboratory.

**The Aryl Hydrocarbon Receptor Mediates Leflunomide-Induced Growth Inhibition of Melanoma Cells**

**Edmond F. O'Donnell [1,2,5], Prasad Rao Kopparapu [1,2,5], Daniel C. Koch [1,2,5], Hyo Sang Jang [1,2,5], Jessica Lynne Phillips [1,3,5], Robert L.Tanguay [2,3,4,5], Nancy I. Kerkvliet [2,4,5], Siva Kumar Kolluri [1,2,3,4,5].** *[1]Cancer Research Laboratory, [2]Department of Environmental and Molecular Toxicology, [3]Molecular and Cellular Biology Program, [4]Environmental Health Sciences Center, [5]Oregon State University, Corvallis, Oregon 97330 USA*

A novel role of the dihydroorotatedehydrogenase (DHODH) inhibitor leflunomide as a potential anti-melanoma therapy was recently reported (Nature 471:518-22, 2011). We previously reported that leflunomide strongly activates the transcriptional activity of the Aryl Hydrocarbon Receptor (AhR) (PLoS ONE 5(10): e13128, 2010). We therefore tested whether the AhR regulates the anti-proliferative effects of leflunomide in melanoma. We first evaluated the expression of AhR in melanoma cells and found that AhR is highly expressed in A375 melanoma as well as in several other cancer cell types. To evaluate whether AhR plays a role in regulating the growth inhibitory effects of leflunomide in A375 cells, we generated a stable cell line from parental A375 cells expressing a doxycycline (DOX) inducible AhR shRNA. Using these cells in the absence or presence of DOX (normal AhR levels or AhR-knockdown, respectively) we found that the anti-proliferative effects of leflunomide, but not its metabolite A771726, were strongly dependent upon AhR expression. It has been well established that supplementation of cells with exogenous uridine completely rescues the anti-proliferative effects due to DHODH inhibition. Thus, we performed uridine rescue experiments in A375 cells to determine whether the anti-proliferative effects of leflunomide are solely due to DHODH inhibition as previously reported. Interestingly, saturating levels of uridine only modestly rescued A375 cells from the anti-proliferative effects of both leflunomide and A771726, indicating additional mechanism(s), apart from DHODH inhibition are responsible for the anti-proliferative effects of leflunomide in melanoma cells. Uridine also did not rescue MDA-MB-435S melanoma cell proliferation after leflunomide treatment. Our results reveal that the AhR is a molecular target of leflunomide and support the feasibility of the clinical application of leflunomide for treating melanoma. Furthermore, analysis of expression data from 967 cancer cell lines revealed that AhR is expressed in multiple different cancer types supporting the intriguing possibility of targeting the AhR for therapy in a number of cancers.

## Using a High Throughput Approach to Assess the Toxicity of TOXcast Chemicals *in vivo*

**Lindsey N. Chalker, Lisa Truong, Robert L. Tanguay**

There is increased concern regarding the potential toxicity of chemicals and compounds in consumer products and in the environment. To address this issue, the U.S. Environmental Protection Agency developed the TOXcast program. TOXcast is designed to help identify and prioritize potentially toxic chemicals, which will permit determination of the biological pathways that cause toxicity. The data collected from TOXcast will be used to develop predictive toxicity models, which will reduce the demand for animal testing in the future. In our lab, we are evaluating both Phase I and II of TOXcast (1078 chemicals) in an *in vivo* model to identify which cause adverse responses. The embryonic zebrafish is an ideal model due to its accelerated developmental growth and its optically clear body throughout development. For each TOXcast chemical, we evaluated morphological and behavioral toxicity. Dechorionated embryos were exposed at 6 hours post fertilization (hpf) to five different concentrations and at 24 hpf, we evaluate their photomotor response using an analysis tool (PRAT). Afterwards, we evaluate the mortality of the chemical at that life stage. At 120 hpf, after the embryonic zebrafish has completed organogenesis, we do complete morphological and behavioral assessments. We evaluated for behavioral defects using Viewpoint Zebrabox to assess the amount of movement the zebrafish exhibited in alternating light and dark periods for 25 minutes. We then conduct morphological evaluations that include 18 morphological endpoints. To date we have completed the assessment of over 700 compounds. Our general approach and initial summary of the results will be presented.

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## A Novel Method for the Detection and Quantitation of Intact Quantum Dots in Tissue Slices

**Josi Herron*, Lisa McConnachie, Dianne Botta, Collin White, Stefanie Schmuck, Jianbo Yu, Russell Dills, Xiaoge Hu#, Xiaohu Gao#, Terrance Kavanagh.**
**Department of Biology, University of Great Falls, Great Falls, MT. # Departments of Bioengineeringand Environmental and Occupational Health Sciences, University of Washington, Seattle, WA*

Semiconductor quantum dots (QDs) are fluorescent nanocrystals composed of a semiconductor core containing heavy metals, such as CdSe or CdTe, surrounded by a stabilizing ZnS cap. Concerns have been raised about their safety and potential toxic effects in biological systems. *In vivo* toxicity studies have attempted to quantitate intact QD in tissues, but currently there is no rapid and cost effective method to do so. Quantitation of intact QD would allow better organ and tissue dose assessments rather than relying on Cd content determination which does not differentiate between degraded or intact QD. To address this, a new method for quantifying tissue QD content was developed. Mice expressing differential levels of glutathione were administered a single 6 µg/kg Cd equivalent dose of TOPO-PMAT QD via intravenous injection. At 1, 8 and 24 hours post-QD administration, mice were euthanized and livers were excised and embedded for histology. QD deposition in liver sections was analyzed by fluorescence microscopy utilizing multispectral imaging/digital image analysis. Mice with depleted glutathione had significantly more QD total fluorescent intensity

relative to mice with normal levels of glutathione. This method will be of great value for determining uptake and degradation rate of QD in a variety of tissues from QD treated mice.

**Media- and Serum-Dependent Uptake of Polymer-Coated Quantum Dots into Hepg2 Cells.**

**Wesley E. Smith, Terry R. Ward, Charlie Corredor**, Rachel Benton**, Collin C. White, Xiaoge Hu*, Xiaohu Gao*, Jonathan D. Posner**, Terrance J. Kavanagh, and David L. Eaton.** Departments of Environmental and Occupational Health Sciences, Bioengineering*, and Mechanical Engineering**, University of Washington, Seattle, WA

The disposition of nanoparticles are influenced by the immediate environmental conditions (*e.g.*, cell culture medium), which can affect interactions with cells. To investigate the influence on environmental conditions with a specific nanoparticle, we measured the uptake of quantum dots (Qdots) coated with poly(maleic anhydride-alt-1-tetradecene), tri-n-octylphosphineoxide (PMAT-TOPO Qdots) into HepG2 cells in combinations of two media: Minimal Essential Medium with alpha modification (MEMα) and Dulbecco's Modified Eagle Medium (DMEM) and two sera: fetal bovine serum (FBS) and NuSerum. HepG2 cells most avidly sequestered PMAT-TOPO Qdots as follows: MEMα/10% NuSerum > MEMα/5% NuSerum > DMEM/10% FBS = DMEM/5% FBS > DMEM/0% serum > MEMα/0% serum. To further investigate the physicochemical characteristics in these various media conditions, we measured the size (dynamic light scattering) and zeta potentials of PMAT-TOPO Qdots in treatment conditions listed above. Our results suggest that optimal uptake is both media- and serum-dependent. Furthermore, uptake efficiency is improved with increasing amounts of NuSerum (5 and 10%), but not with FBS suggesting a serum-dependent effect. Current experiments are directed at elucidating the underlying biophysicochemical interactions governing the different levels of uptake.

**Using RNA-Seq to Define Gene Expression Responses Following Gold Nanoparticles (Aunps) Exposures in Embryonic Zebrafish**

**Lisa Truong [a,b], Susan C. Tilton[c], Tatiana Zaikova [b,d], Katrina M. Waters[c], James E. Hutchison[b,d] and Robert L. Tanguay[a,b].** *[a] Department of Environmental and Molecular Toxicology, The Sinnhuber Aquatic Research Laboratory and the Environmental Health Sciences Center at Oregon State University, Corvallis, OR; [b] The Safer Nanomaterials and Nanomanufacturing Initiative, Oregon Nanoscience and Microtechnologies Institute; [c] Computational Biology and Bioinformatics Group, Pacific Northwest National Laboratory, Richland, Washington 99352, USA; [d] Department of Chemistry and the Materials Science Institute, University of Oregon, Eugene, Oregon 97403, USA*

In a previous study, we assessed the interaction between nanoparticles and biological systems using a library of gold nanoparticles (AuNPs) and the sensitive embryonic zebrafish model. We found that AuNPs functionalized with 3-mercaptoproponic acid (3-MPA) and 2-(2-(2-mercaptoethoxy)ethoxy)ethanol (MEEE) induced differential biological responses. Embryonic zebrafish exposed to 1.2 nm 3-MPA-AuNPs failed to respond to a touch on the caudal fin at 120 hours post fertilization, while those exposed to 1.5 nm MEEE-AuNPs had a normal touch response. To investigate the molecular mechanism underlying the differential touch response, whole animal RNA-seq was conducted. Total RNA was isolated from

embryonic zebrafish exposed to the 100% effective concentration (EC100) for 1.2 nm 3-MPA- and 1.5 nm MEEE-AuNPs (10 ppm) at 48 hours post fertilization (hpf). While the core materials (Au) for both nanoparticles are identical, the surface functionalities caused unique gene expression changes. 57,095 transcripts were mapped to ensembl version 9. At 48 hpf, 810 transcripts were common between MPA- and MEEE-AuNP exposed groups, while 1,099 and 1,523 transcripts were unique and statistically significantly differentially expressed in MPA- and MEEE- exposed embryos, respectively. Bioinformatic pathway analysis similarly identifies unique pathway perturbations following exposure to these AuNPs.

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### Quantifying Quantum Dots in Frozen Tissue Sections using Autometallography

**Collin White, Christopher Schaupp, Josi Herron, David Scoville, Lisa McConnachie, Dianne Botta, Jianbo Yu, Russell Dills, Xiaozhong Yu, Xiaoge Hu*, Xiaohu Gao*, Jasmine Wilkerson, and Terrance Kavanagh**. *Departments of Environmental and Occupational Health Sciences and *Bioengineering University of Washington, Seattle, WA 98195*

Quantum dots (QDs) are engineered nanoparticles frequently composed of a CdSe core, ZnS shell, and an assortment of polymer coatings specific to their application. QDs are used in electronic systems because of their semiconductor properties and in biomedical research and medicine as imaging tools because of their unique fluorescent properties. Their widespread use and heavy metal core composition have raised concerns about their safety. An important consideration in evaluating QD toxicity is the accurate quantification of these nanoparticles within tissues. The current measurement methods favored include inductively coupled plasma mass spectrometry (ICP-MS) for the metal components of QDs, and QD fluorescence directly in tissue sections using microscopy. However, ICP-MS is expensive and cannot distinguish between metals present in QDs or free ions, and fluorescence microscopy is often difficult because of interfering tissue autofluorescence. We adapted a silver-enhanced autometallography technique for detecting QDs in frozen tissue sections. This technique is efficient and inexpensive, and quantification using digital imaging and densitometry correlates well with direct QD fluorescence measurements. The ability to efficiently measure QDs in tissues will provide important dose information that can be useful for evaluating the adverse health effects of QD exposures.

### Absence of Upstream Consensus Regulatory Element Sequences for NRF2 in Human Glutathione *S*-transferase Genes

Christopher M. Schaupp, Theo K. Bammler, Richard P. Beyer, Terrance J. Kavanagh, and David L. Eaton. *Center for Ecogenetics and Environmental Health, University of Washington, Seattle, WA*

Nuclear factor erythroid-derived 2-like 2 (NFE2L2, or NRF2) is involved in antioxidant response to cellular stress. In response to oxidative or electrophilic stimuli, NRF2 binds its target genes containing an antioxidant response element (ARE), upregulating expression of detoxifying/antioxidant enzymes. The phytochemical sulforaphane (SFN) is an effective inducer of many GSTs in rodents, and may have therapeutic value based on its ability to activate NRF2-mediated antioxidant pathways. However, human studies *in vivo* and *in vitro*

have not generally seen a robust induction of GSTs following SFN administration. To identify the potential basis for a possible inter-species difference in GST inducibility, we used the web-based oPOSSUM software to identify ARE sequences up to 10000 base pairs upstream of the transcriptional start sites of antioxidant enzymes putatively regulated by NRF2 in murine and human genomes. Only three out of 19 human GST genes contained an ARE consensus sequence within 10kb of the transcriptional start site, whereas ARE sequences were present in 11 of 17 murine GSTs analyzed. Our data suggest that inter-species differences in GST inducibility may result from differences in ARE location and sequence upstream of murine and human GST genes—a conclusion which may have major implications in the clinical setting.

**Development of an Assay to Examine Learning and Memory in Adult Zebrafish**

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The number of neurodegenerative diseases has markedly increased in the past decade. One of the initial signs of neurodegeneration is a deficit in memory. Of the various causes of neurodegeneration, environmental exposure to contaminants likely plays a role in age-related memory loss. For example, Bisphenol A (BPA), an estrogenic chemical used in plastics and resin-lined canned goods, may be a factor in memory loss. Current developments in behavioral assays for learning and memory are relatively low-throughput and time intensive. Zebrafish present a useful model to examine memory and learning due to their rapid development, external dosing, and ability to conduct large studies in short time frames. In this study, we developed a passive avoidance shuttle-box assay measuring adult zebrafish memory. The shuttle-box consisted of a tank divided by a barrier with an opening to allow passage. Upon a light stimulus, the animals had to cross the barrier or receive a minor shock (3.5V). Learning was measured as a function of the time to decide to cross the barrier. Adult zebrafish exposed to 0.01μM BPA showed a decrease in learning ability compared to controls, presenting a potential role in neurodegeneration. These studies were sponsored by NIH P30ES00210, 1R21ES018970, and 5T32ES007060.

**Controlled Particle Dispersion of Silver Nanoparticle and Toxicity Assessment in the Embryonic Zebrafish: Size and Surface Coating Dependent Toxicity**

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The strong aggregation tendency of silver nanoparticles (AgNPs) complicates the elucidation of their toxic potential. To investigate size and surface coating dependent toxicity, we exposed zebrafish embryos to AgNPs of 20 and 110 nm core size that were surface coated with either citrate or polypyrrolidone (PVP). We also investigated these materials in solution media of varying ionic strength in an attempt to alter particle dispersion. Zebrafish embryos developed normally in the low ionic strength environments of 62.5 μM $CaCl_2$ ($CaCl_2$) and ultrapure water (UP). AgNPs were well dispersed in $CaCl_2$ and UP, but in standard zebrafish embryo medium (EM) the hydrodynamic diameter was markedly increased. Exposure of embryos to AgNPs suspended in $CaCl_2$ and UP caused higher developmental toxicity than the suspensions in

EM. Embryonic toxicity in UP was the same as in $CaCl_2$ which likely resulted from stable AgNPs dispersion and increased bioavailability. We found that 20 nm AgNPs were more toxic than 110 nm AgNPs, and the PVP coated materials were more toxic than the citrate coated AgNPs. However, silver tissue burden was positively correlated with the degree of toxicity only under $CaCl_2$ conditions. Our results demonstrate that toxicity of AgNPs and silver tissue burden was strongly correlated to test medium, and thus the consideration of ionic environment for experiments is critical in the toxicity evaluation of AgNPs. Additionally, no other model but the embryonic zebrafish offered these experimental power and flexibility to identify potential toxicity of AgNPs.

### Exploring the Relationship between Expression of the Chemokine Receptor CXCR4 and Suppressed Tumor Metastasis in Alcohol-Drinking Mice

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Alcohol consumption is a known risk factor for developing primary breast cancer. However, metastasis of breast cancer is the main cause of mortality, and very little is understood about the effects of alcohol on cancer progression. Prior studies in our laboratory showed that high alcohol consumption decreases mammary tumor metastasis to the lung. One potential explanation for this reduction involves expression of the chemokine receptor CXCR4, which plays an important role in migration of cancer cells. We hypothesized that chronic alcohol consumption decreases expression of CXCR4 on tumor cells, thus impairing their ability to extravasate into peripheral sites. Mice were provided with 18% w/v alcohol in drinking water, beginning 6 weeks prior to orthotopic injection of 4T1.2 mammary tumor cells. Western blot analysis of primary tumor homogenates revealed that CXCR4 expression was significantly reduced in primary tumors by up to 80%. However, no correlation was observed between CXCR4 expression in the primary tumor and number of lung metastases in individual animals. Additional experiments showed no direct effect of ethanol treatment on expression of CXCR4 on tumor cells *in vitro*. Further investigation is necessary to determine the significance of decreased CXCR4 in alcohol-mediated suppression of tumor metastasis.

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