14th Annual U of S Undergraduate Toxicology Symposium

Saturday April 1st, 2017

9:00AM – 2:00PM

WCVM Room 2104

14th Annual Undergraduate Toxicology Symposium Schedule
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TIme	Speaker	Topic Title
9:05AM – 9:20AM	Jordan Gelowitz	The scary intoxication of strychnine
9:20AM – 9:35AM	Robyn Akre	Juvenile soil invertebrate avoidance to soil contaminants
9:35AM – 9:50AM	Madison Blischak	Imidacloprid: Effects on honey bees and contribution to colony collapse disorder
9:50AM – 10:05AM	Carly Colville	Use of endpoint specific rates of development to determine acute and sub-chronic toxicity of emerging contaminants to early life stages of *Salvelinus namaycush*
10:05AM – 10:20AM	Arashpreet Natt	The impact of contraceptives on amphibians in aquatic environments
10:20AM – 10:35AM	Benjamin Abelseth	Examining the adverse effects of hormone replacement therapy in transgender patients
10:35AM – 11:00AM	Coffee break	
11:00AM – 11:15AM	Darja Besermenji	Impact of herbicides garlon XRT, garlon RTU and their active ingredient in two northern soils
11:15AM – 11:30AM	Stephanie Vuong	Determining the influence of blood lipid levels and plasma storage stability on the interpretation of blood cannabinoid levels
11:30AM – 11:45AM	Yu Su	The impact of microplastics on zooplankton
11:45AM – 12:00PM	Brie Chiasson	Use of spider venom to manage chronic pain
12:00PM – 12:15PM	Justine Burant	Toxic effects of water hemlock in cattle and management practices which can be used to eradicate it from rangelands
12:15PM – 1:15PM	Lunch	
1:15PM – 1:30PM	Esther Lin	Tissue partitioning and genotoxic potential of diamondoids in rainbow trout
1:30PM – 1:45PM	Darian Erman-Pollock	The role of oxidative stress and the gut microbiome in the development of autism spectrum disorder
1:45PM – 2:00PM	Anureet Tiwana	Effects of organic UV filters on steroidogenesis in the human adrenocortical carcinoma cell line, H295R
2:00PM – 2:15PM	Nancy Gill	Towards improved methods for determination of aldehyde oxidase activity in fish
2:15PM – 2:30PM	Laith Alphin	Assessment of the health benefits of selenium and polyunsaturated fatty acids in offsetting mercury toxicity from fish in the athabasca and slave rivers
2:30PM – 2:45PM	Andre Coutu	Mercury case study: grassy narrows

The Scary Intoxication of Strychnine

Jordan Gelowitz

This article will examine strychnine poisoning through the explanation of acute exposure effects. Its mechanism of action is inhibiting glycine receptors in the spinal cord and brain stem regions causing stimuli hypersensitivity. Strychnine is a strong convulsant rodenticide agent, making it dangerous to humans and animals. Non-target species poisoning commonly occur when animals eat the seeds designed to poison rodents. Analysis of strychnine poisoning of canines in western Canada has show both a high mortality that occurs in as little as 30 minutes. This chemical has been used in China as an herbal medicine to treat muscle pain, which has lead to mild poisoning in some cases. Human strychnine poisonings although rare, are often serious cases that required immediate and intensive emergency care management to survive. The purpose of becoming informed about strychnine is to understand the hazard and exposure to treat/prevent poisonings.

Keywords: Strychnine, mortality, poisoning, emergency

Juvenile soil invertebrate avoidance to soil contaminants

Robyn Akre, Amy Gainer, Steven Siciliano

Avoidance tests are commonly used to evaluate the response of soil organisms to contaminated soils. Due to their ease of use and low cost, these tests have become increasingly popular in soil ecotoxicology testing. Existing literature on avoidance of soil invertebrates mainly focuses on adult organisms and lacks data on juvenile responses. However, the sensitivity of juveniles to contaminated soils can influence the habitat range of populations and influence the ability of soils to provide ecosystem services. The objectives of this study were to characterize the avoidance of juvenile soil invertebrates to three contaminants (phenanthrene, copper sulfate, and sodium chloride) and compare responses to published literature for responses of adult species. Three species of soil invertebrates, *Folsomia candida (F. candida), Eisenia fetida (E. fetida), and Enchytraeus crypticus (E. crypticus)* were tested with each of the contaminants. Artificial soil was used for each experiment and each experiment ran for 48-72 hours. Net response to the contaminant was calculated to determine if there was an avoidance or an attraction response. Preliminary data for copper sulfate suggests that *E. fetida* shows avoidance, but at higher concentrations than adult avoidance. *F. candida* showed a lack of preference for either clean or contaminated soil up to concentrations of 10000 mg/kg. There is no literature yet on the adult *F. candida* avoidance of copper sulfate, but 10000 mg/kg is well above the threshold for reproductive effects in collembolan species.

Key Words: Avoidance Response, Collembola, Earthworms, Metals, Organic Chemicals

Imidacloprid: Effects on Honey Bees and Contribution to Colony Collapse Disorder

Madison Blischak

Honey bees play an important role in the ecosystem, society, and economy. They are pollinators that many plants depend on for cross-pollination, and indirectly keep beef and dairy industries afloat through pollination of commercial crops. Honey bees also provide nutrition to our society and contribute to the economy through the production and sales of honey. However, honey bees are experiencing a phenomenon known as colony collapse disorder that could result in endangerment of this beneficial species. Colony collapse disorder occurs when worker bees in a colony disappear, leaving the queen bee behind with the younger bees and a few nurse bees. A broadly used neonicotinoid insecticide, imidacloprid, is thought to be responsible. Imidacloprid is a systemic seed treatment that can be transferred to honey bees when bees forage on crops treated with imidacloprid. As a nicotinic acetylcholine receptor agonist, imidacloprid has been shown to affect the cholinergic signaling in honey bees, which can lead to abnormal behavior. This talk will highlight recent studies that have examined specific effects of imidacloprid on exposed honey bees, such as impaired aversive learning and memory retention, and impaired decision-making. With the broad and increasing use of imidacloprid, and decreasing numbers of honey bees, these studies emphasize that there is a need to reconsider current pesticide risk assessment in hopes of saving our economy, society, and ecosystem.

Key words: neonicotinoid, aversive learning, pesticide, decision-making

Use of Endpoint Specific Rates of Development to Determine Acute and Sub-Chronic Toxicity of Emerging Contaminants to Early Life Stages of *Salvelinus namaycush*

Colville, C., Alcaraz, A., Schultz, D., and Hecker, M

Emerging contaminants (ECs) are substances that are only recently gaining a reputation in scientific and public communities as potential threats to the health of both humans and the natural environment. They are an issue for northern and rural Canadian communities where waste water treatment technology is lacking. ECs can be variety of chemicals, including pharmaceuticals, personal care products, and flame retardants. Current data on their toxicity is inadequate, especially for native fish species of cultural, economic, and recreational relevance such as lake trout (*Salvelinus namaycush*). Four specific chemicals that are of particular concern are two pharmaceuticals: 17α-ethinylestradiol (EE2), a potent synthetic estrogen, fluoxetine (FLX), a common antidepressant, and two flame retardants: hexabromocyclododecane (HBCD) and a short chain chlorinated paraffin (SCCP). This study aimed to investigate the acute and sub-chronic toxicity of these four ECs on developmental endpoints in early life stages of lake trout, from fertilization to swim-up. Lake trout were exposed to EE2, FLX, HBCD, and SCCP at a range of six concentrations, the lowest being environmentally relevant. The duration of the exposure began immediately after fertilization and continued after swim-up. Endpoints of interest include fertilization success, embryonic development, hatchability, fry survival, and transition to exogenous feeding. We hypothesized that exposure to these ECs would have concentration-dependent effects on each of these endpoints. Results will help us gain a better understanding of the potential hazards these chemicals pose to this species with the long term aim to generate information that will help to appropriately protect aquatic ecosystems.

Keywords: wastewater treatment, pharmaceuticals, flame retardants, developmental effects

The Impact of Contraceptives on Amphibians in Aquatic Environments

Arashpreet Natt

Pharmaceuticals and personal care products (PPCPs) are being found in water bodies around the globe. Human consumption of pharmaceuticals (i.e. birth control pills), and the use of cosmetic products (i.e. fragrances, sunscreens, makeup etc.) have led to the contamination of these water bodies. Unfortunately, water treatment plants do not facilitate the removal of PPCPs, so these chemicals are being released into aquatic environments. Ethinylestradiol (EE2), a synthetic hormone used in birth control pills, is only one of a cocktail of natural and synthetic hormones that humans excrete into wastewater, including other estrogens. EE2 has a potent biological effect at low levels, which has been shown to create "intersex" in amphibians. However, this compound is very difficult to remove from wastewater, but it can be present in high concentrations in aquatic environments. This presentation will focus on severe cases of EE2 contamination, which has resulted in the feminization of amphibian species. Amphibian feminization is problematic due to the transient life cycle of many amphibians and the potential for multi-generational effects. The model species, *Xenopus Laevis* (African Clawed Frog), will be focused on to provide a review of the literature concerning feminization events in these species in response to EE2 exposure.

Keywords: PPCP, *Xenopus Laevis*, Feminization, Demasculinization, EE2.

Examining the Adverse Effects of Hormone Replacement Therapy in Transgender Patients

Benjamin Abelseth

In ecotoxicology, sex differentiation and reversal is a major focus when looking at environmental contaminants, notably endocrine disrupting compounds. In a clinical setting for transgender individuals, sex reversal is instead the goal of hormone treatment. In this context, my presentation will look at the pharmaceuticals used in hormone replacement therapy (HRT), their intended effects, and any potential adverse health outcomes in transgender individuals. For female to male transgender patients, the primary goal of care is the induction of male secondary sex characteristics using testosterone therapy. Among the negative effects, severe liver toxicity, erythrocytosis, and increased occurrence of breast and uterine cancers have been highlighted. For male to female transgender patients, treatment is more complicated, with both anti-androgen and estrogenic therapies being employed. Adverse effects seem to be more prominent, with venous thromboembolism being of note, as well as liver toxicity and coronary artery disease. Overall, the positive physical and mental outcomes of HRT in transgender individuals far outweigh any undesirable effects. However, the lifelong administration of HRT still poses a unique risk to a vulnerable population, and the toxic effects of these treatments should not be ignored.

Keywords: Transgender, hormone therapy, adverse effects, sex reversal

Impact of herbicides Garlon XRT, Garlon RTU and their active ingredient in two Northern soils

Darja Besermenji, Katherine Stewart

Herbicides and their toxic capabilities have been the subject of extensive study; however, how these products impact boreal soils, vegetation, and wildlife in northern climates has received little attention. Recent proposals to apply herbicides Garlon RTU and Garlon XRT by SaskPower and Yukon Energy Corporation in northern Saskatchewan and Yukon Territory, has been met with opposition from northern communities. Providing site specific and community driven investigations regarding the impacts of Garlon RTU and XRT are important for supporting informed, collaborative decision making between utilities and northern communities. The objective of this study was to determine and compare the median effective concentration (EC_{50s}) for standard enchytraeid survival and reproduction toxicity tests in two northern soils (Hanes Junction 2 and Little Salmon) dosed with Garlon XRT, and Garlon RTU. The definitive test was completed after 28 days, in which the survival of parental *Enchytraeus albidus* worms and the fecundity (number of juveniles) was measured. Modelling of dose response relationships for adult survival and reproduction endpoints in each soil and herbicide component was completed. These results not only provide us with insight into what the degree of toxicity of these herbicides is, but they are also an important tool that will be used to inform northern communities on whether there is a need for concern.

Determining the Influence of Blood Lipid Levels and Plasma Storage Stability on the Interpretation of Blood Cannabinoid Levels

Stephanie Vuong, Muath Hilal, Dr. Jane Alcorn, Dr. Andrew Lyon

Medical marijuana can be used as a treatment for pediatric patients diagnosed with epilepsy. The appropriate dosage of medical marijuana needs to be developed to ensure benefits are being maximized while the risks are being minimized. Factors, such as the storage of blood in the blood sample collection tubes and the variability in the patients' blood characteristics, can influence the cannabinoid levels in the blood. Therefore, we determined the influence of blood sample collection tubes on the cannabinoid stability as well as determined the unbound fraction of bioactive cannabinoids in plasma collected from healthy participants compared to patients on a ketogenic diet by using our LC-MS/MS assay. 6 different types of blood sample collection tubes were tested for any interference with the cannabinoids by spiking the plasma with cannabinoids at three different concentrations, low QC (quality control samples are used to show stability) (2.53ng/mL), mid QC (50.56ng/ml), and high QC (101.2ng/mL). Quality controls are used to ensure that the results of our analysis are consistent and accurate. Using the LC-MS/MS assay, we can quantify the cannabinoid levels by measuring the mass-to-charge ratios. The tube with the closest mass-to-charge ratio to the control tubes shows the least interference, making it the ideal tube to use in the clinical trials. Ultrafiltration was used to measure the unbound/bound fraction of cannabinoids. Cannabinoids that are not bound to the blood lipids will pass through the ultrafiltration device, allowing us to measure the fraction. It was determined that the higher the blood lipid level was, the lower the fraction of unbound cannabinoids. Therefore the medical marijuana would have less of an effect on individuals with high blood lipid levels.

Keywords: Cannabinoids; plasma; epilepsy; LC-MS/MS; ketogenic

The Impact of Microplastics on Zooplankton

Yu Su

Microplastics are small plastic particles that generally range from 1 to 5 mm diameters. In recent years, there has been growing environmental concern regarding microplastics since they are widespread and persistent in the aquatic environment at high levels, especially in marine ecosystems. The impact of microplastics on marine organisms (i.e. mussels, worms, fish and seabirds), has been widely reported. However, the impacts of these contaminants on zooplankton remain under researched. As an important primary producer in the marine food web, zooplankton feed a range of aquatic animals; therefore, it is important to determine the effects microplastics may be having on zooplankton.

This study showed that the ingestion of microplastics by zooplankton in the marine ecosystem was feasible. The potential impacts of microplastics include influencing health and function of the zooplankton (i.e. reduced algal feeding, small eggs with decreased hatching success, reduced survival due to declining energetic reserves), trophic transferring contaminants to predators, and potentially repercussion on the marine nutrients influx (excreting faecal pellets containing microplastics, impacting their properties and sinking rate). The findings confirm that ingestion of microplastics of zooplankton can harm the marine ecosystem.

Keywords: Microplastics, contaminants, zooplankton, predators, nutrients influx

Use of Spider Venom to Manage Chronic Pain

Brie Chiasson

Chronic pain is an ailment that 1 in 5 people suffer from worldwide with little relief given from common pharmaceuticals. The desire to relieve pain has risen exponentially and research has begun analyzing spider venom in hopes of finding a treatment. Spider venom, used to capture prey and divert predators, is composed of a cocktail of proteins that block nerve activity. Insects only express one sodium channel subtype that is sensitive to most sodium channel modulators, as opposed to humans who have multiple sodium channel subtypes that control different aspects of the body. Human voltage-gated sodium channels (Na_V) have eight subtypes: 1.1 to 1.9. The most important subtype for chronic pain is $Na_V1.7$. If the $Na_V1.7$ channel is blocked the brain does not receive signals from the source of pain, essentially turning off the pain. Using the ArachnoServer and CLC sequence viewer to look at and compare the sequence of spider venom peptides, tarantulas (*Theraphosidae*) were found to have more specificity to $Na_V1.7$ resulting in pain-relieving effects seen in rats. A better understanding of spider venom specificity of the Na_V channels is critical to alleviate chronic pain while avoiding unwanted side effects.

Keywords: Tarantulas (*Theraphosidae*), analgesic, voltage-gated sodium channel ($Na_V1.7$)

Toxic effects of water hemlock in cattle and management practices which can be used to eradicate it from rangelands

Justine Burant

Water hemlock (Cicuta spp.) is a biennial plant located along water ways of agricultural fields. The plant naturally produces a deadly neurotoxin called cicutoxin, which is located in its roots and tubers. Water hemlock is of particular concern to cattle producers because cattle commonly feed near streams and rivers and are attracted to the smell of these plants. The entire plant can be easily pulled out of moist soil so cattle will often consume the whole plant which increases the chances of poisoning. Cicutoxin acts as a GABA-receptor antagonist which produces a stimulatory effect on the central nervous system. Upon ingestion this compound can induce muscle twitching, tachycardia, increased salivation, dilated pupils, and convulsions. Death due to respiratory paralysis is the typical end result. There is no specific cure for water hemlock toxicity. It is widely spread across North America so its important that cattle producers take the proper steps to eradicate it from their land. This can be done by physically removing the plants or by blocking off the area, or through the use of herbicides like 2,4-D or glyphosate. Understanding the toxin's mechanism of action and proper plant removal methods will result in less cattle death.

Keywords: cicutoxin, herbicide control, poisonous plant, cowbane

Tissue partitioning and genotoxic potential of diamondoids in rainbow trout

Lin,Y, Chen, Y, Raine, J

Oil sands extraction along the Athabasca River (AB, Canada) has potential environmental impacts on the surrounding ecosystem related to air emissions, water use, wastewater production, potential groundwater contamination, and land and habitat disturbances. The wastewater, which contains residual bitumen, is called Oil Sands Process-affected Water (OSPW). A new group of naphthenic acid, diamondoids, have been identified in OSPW and weathered crude oil. Previous studies in marine mussels have determined the concentrations likely to induce sublethal effects. The aim of this study was to determine the uptake and tissue partitioning, metabolism and genotoxicity of three diamondoids (1-Adamantanecarboxylic Acid, 3, 5, 7-Trimethyladamantane-1-carboxylic acid) in rainbow trout. Fish were exposed to 0, 0.06 µM, 0.6µM and 6µM of each chemical for 96 hours. Results obtained allow us to achieve a more comprehensive view of the toxicity on aquatic organisms of diamondoids.

Keywords: naphthenic acid, diamondoids, genotoxicity,

The Role of Oxidative Stress and the Gut Microbiome in the Development of Autism Spectrum Disorder

Darian Erman-Pollock

Autism spectrum disorder is a neurodevelopmental disorder characterized by a range of cognitive, social and verbal impairments. Despite vast research conducted on autism spectrum disorder, the understanding of this disorder, along with effective treatment methods are limited. Furthermore, prevalence of autism is drastically increasing. It is therefore important to gain a better understanding of autism and its causes. A new hypothesis describes how oxidative stress and sulfur metabolism deficiencies could alter the gut microbiome, leading to increased gut permeability. Ultimately, compounds that are normally restricted in the gut could cross the blood brain barrier causing neuroinflammation and autism spectrum disorder. This talk will describe how oxidative stress, environmental contamination and, or genetic predispositions can lead to autism spectrum disorder, and will focus on how the gut microbiome is changed, how this change can lead to microbial toxin production and ultimately, how these toxins play a role in the development of autism spectrum disorder.

Keywords: metabolic deficiencies, gut permeability, microbial toxins, neuroinflammation

Effects of organic UV filters on steroidogenesis in the human adrenocortical carcinoma cell line, H295R

Anureet Tiwana

Society is developing and using an ever-growing number of new chemicals. In the past, monitoring was limited to a small number of environmental priority pollutants. However, recent advances in analytical chemistry have enabled scientists to detect these chemicals of emerging concern (CECs) in the environment with unprecedented sensitivity. One CEC that has recently gained the attention of scientists and regulators are UV filters as they are detected in the environment with increasing frequency. Organic UV filters, which are also called chemical filters, are usually aromatic compounds with carbonyl groups. Because of their high lipophilicity and poor biodegradability, organic UV filters often end up in sewage sludge during wastewater treatment, or accumulate in sediments and biota. The main objective of the proposed research is to test whether benzophenones and their metabolites have endocrine disrupting properties. We specifically focused on the effects of these CECs on hormone production using the H295R steroidogenesis assay. The effects of six selected organic benzophenone UV filters and their metabolites that have been prioritized by the United States Environmental Protection Agency (Benzophenone-3 (BP-3), Benzophenone-4 (BP-4) and their chlorinated compounds) will be investigated using H295 cells. Specifically, we assessed the effects of these compounds on (1) cell viability, (2) hormone production, and (3) the abundance of transcripts of selected steroidogenesis-related genes in H295R cells. ELISA tests were performed to determine the effect of benzophenones and their chlorinated compounds on steroidogenesis. According to the literature, benzophenones can estrogenic or anti-estrogenic.

Keywords: H295R, UV filters, steroidogenesis, benzophenones

Towards improved methods for determination of aldehyde oxidase activity in fish

Nancy Gill, Markus Brinkmann

Ongoing research has characterized the important role of aldehyde oxidase (AOX) in detoxifying therapeutic drugs and contaminants. However, research pertaining to AOX activity in non-mammalian organisms is lacking. The aim of this research project was to develop and validate methods to characterize AOX activity, and to use these methods to assess AOX activity in different fish species. A novel assay was developed that quantifies the conversion of the exogenous aldehyde substrate 4-(dimethylamino)cinnamaldehyde (DMAC) to its corresponding fluorescent acid by AOX in partially purified cytosol from rainbow trout (*Oncorhynchus mykiss*) following ultracentrifugation. The absence of CYP-mediated biotransformation reactions in cytosolic fractions was confirmed by quantifying 7-ethoxyresorufin *O*-deethylase (EROD) activity, as a prototypic microsomal activity, in both cytosol and microsomes. Michaelis-Menten kinetic parameters (K_m, V_{max}) of the observed reaction were comparable to those reported for assays which photometrically monitored the decrease of substrate concentration. An inhibition study with hydralazine, a selective inhibitor of mammalian AOX, revealed similar inhibition constants (K_i). The assay is of great potential use in pharmacological research because we successfully applied it to partially purified human and rat cytosol. Because the assay quantifies the increase of product rather than the decrease of substrate, it could potentially be used for *in vitro* experiments with permanent cell lines without the need to purify the cytosol. We were able to establish a sensitive and rapid assay for determination of AOX activity in fish. This procedure can be applied in future studies to broaden our knowledge on the relative importance of this class of enzymes.

Keywords: Toxicokinetics, biotransformation, molybdenum oxidases.

Assessment of the Health Benefits of Selenium and Polyunsaturated Fatty Acids in Offsetting Mercury Toxicity from Fish in the Athabasca and Slave Rivers

Laith T. Alphin, Tim Jardine

Mercury (Hg) can readily move between environmental compartments and biomagnify in food webs, making it a long-standing environmental and human health concern. Consumption of fish is the main route of exposure for mercury in people and those that come from lakes and rivers come with greater mercury burden. Hence, consumption advisories are often present at lakes and Health Canada has put a retail limit of 0.5 ppm mercury on most fish. However, guidelines do not consider whether the benefits of eating fish outweigh the detriments and how the presence of selenium may offset toxicity. My objective was to determine if current Canadian health guidelines for fish consumption are protective for general and sensitive populations when accounting for the mercury binding properties of selenium and the health benefits associated with n-3/n-6 polyunsaturated fatty acids (PUFA). Whitefish, goldeye, burbot, northern pike and walleye were obtained from 5 locations in the Athabasca and Slave River region. Mercury and selenium analysis were obtained from a previous study (n = 622), where they were analysed for trace metals by ICPMS. PUFA concentrations were analyzed in subsets of the fish using gas chromatography mass spectrometry. Based on preliminary analysis, most samples have large selenium health benefit value (Se-HBV), suggesting selenium in the fish is protective against mercury toxicity. The Se-HBV was used determine the risk of mercury toxicity. PUFA concentrations were used in estimating the benefit/risk of coronary heart disease in adults and visual recognition memory in children.

Keywords: PUFA, Se-HBV, Athabasca, LTA, visual recognition memory, coronary heart disease,

Mercury Case Study: Grassy Narrows

André Coutu

Anthropogenic activities have increased the emission of mercury in to the environment. Canada is among the worst in this regard, and their mercury emissions have increased greatly. Methylmercury is known to bioaccumulate and biomagnify in freshwater organisms making it a contaminant of concern to Canadian aquatic systems. Methylmercury is highly neurotoxic to humans and therefore consumption should be monitored and limited. A specific case of chronic exposure to methylmercury in Canada is the residents of grassy narrows, Ontario. A chlor-alkali plant was responsible for mercury containing waste products being released in to nearby rivers. The fish are then contaminated with the mercury after activation to methylmercury, by microorganisms. The residents who rely on them as a source of food then consume the fish. Methylmercury concentrations in fish caught in the area were found to be much higher than the governmental guidelines. As of now reclamation of the river has not yet began. A range of symptoms have been felt by the population from difficulty swallowing to more significant neurological effects such as loss of motor function.