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Laurentian SETAC 24th

CONFERENCE & AGM

University of Ottawa | Ottawa, ON
June 7, 2019

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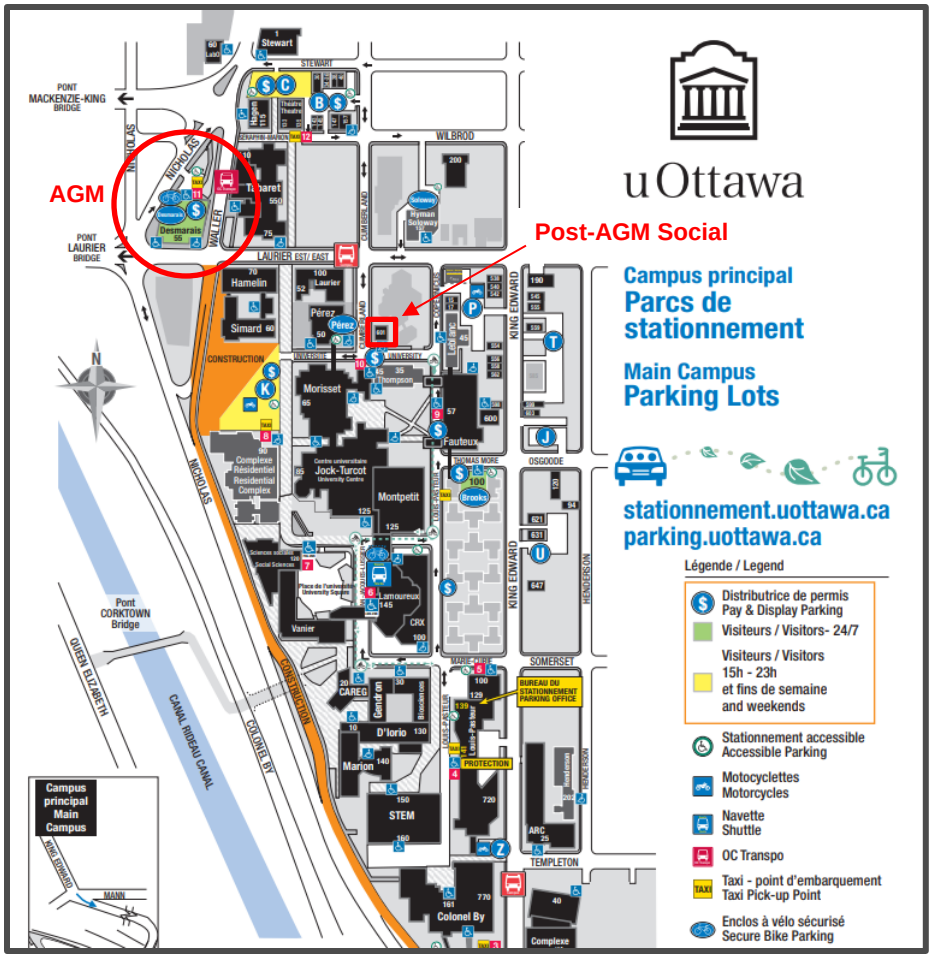

AGM and Conference:
Desmarais Hall, University of Ottawa
55 Laurier Avenue East

Post-AGM Social:
Café Nostalgica
601 Cumberland Street

CONFERENCE PROGRAM

Friday, June 7th 2019

Time	Location	Schedule Item
8:30 – 9:00	**Room 4101**	**Registration** Poster Set-up
9:00 – 9:15	**Room 1130**	**Opening Remarks** *Oana Birceanu, L-SETAC President*
9:15 – 9:30		**SETAC North America Update** *Ryan Prosser, SETAC North America Representative*
9:30 – 10:15		**Investigating environmental contaminants of concern to support decision-making: from the St. Lawrence to the Arctic** ***Dr. Magali Houde*** *Research Scientist, Environment and Climate Change Canada and Adjunct Professor, Université du Québec à Montréal*
10:15 – 11:00	**Room 4101**	**Posters and Morning Break** (Student Poster Judging)
11:00 – 11:45	**Room 1130**	**How research and regulatory risk assessment have advanced policy making in Canada over the last decade** ***Dr. Angelika Zidek*** *Senior Manager, Healthy Environments and Consumer Safety Branch, Health Canada*

CONFERENCE PROGRAM

Friday, June 7th 2019

Time	Location	Schedule Item
11:45 – 12:30	**Room 1130**	**Annual General Meeting**
12:30 – 2:00	**Room 4101**	**Buffet Lunch** & Mentoring Activity
2:00 – 2:15	**Rooms 1130 & 3105**	**Concurrent Platform Presentations** ***Sessions A & B***
2:15 – 2:30		
2:30 – 2:45		
2:45 – 3:00		
3:00 – 3:45	**Room 4101**	**Posters and Afternoon Break** (Student Poster Judging) **& Book Draw**
3:45 – 4:00	**Rooms 1130 & 3105**	**Concurrent Platform Presentations** ***Sessions C & D***
4:00 – 4:15		
4:15 – 4:30		
4:30 – 5:00	**Room 1130**	**Student Judges Meet**
	Room 4101	Poster Take-down
5:00 – 7:00	**Café Nostalgica** 601 Cumberland Street	**Student Awards Social & Dinner**

Platform Presentations

Time	Session A: Emerging Contaminants of Environmental Concern Room 1130
2:00 – 2:15	**Emerging polyfluoroalkyl substances (PFAS) metabolize to bioactive products that modify proteins and promote cell toxicity** Rand, Amy Department of Chemistry, Carleton University, Ottawa, ON
2:15 – 2:30	**The effect of celecoxib on carbon tetrachloride induced liver toxicity** Harris, Todd R.[1], Sean Kodani[2], Amelia Rand[1], Jun Yang[2], Denise Imai[3], Sung Hee Hwang[2], Bruce Hammock[2] [1]Department of Chemistry, Carleton University, Ottawa, ON; [2]Department of Entomology and Nematology, University of California, Davis; [3]Comparative Pathology Laboratory, School of Veterinary Medicine, University of California, Davis
2:30 – 2:45	**Toxicity of novel fire suppression gels to plant germination and emergence** *Graetz, Sarah, Paul Sibley, Ryan Prosser School of Environmental Sciences, University of Guelph, Guelph, ON
2:45 – 3:00	**Identification of targets of aryl phosphate flame retardant toxicity in the rat liver ovary and adrenal glands** Wade, Michael[1], Alice Kawata[1], Marybeth Creskey[2], Terry Cyr[2] [1] Environmental Health Science & Research Bureau, Health Canada; [2]Centre for Biologics Evaluation, Biologics and Genetic Therapies, Health Canada

*** Student presentation**

Platform Presentations

Time	Session B: Field Impacts of Toxicants in Aquatic Systems Room 3105
2:00 – 2:15	**Source Determination of Polycyclic Aromatic Compounds for In-Situ Oil Sands Operations in Cold Lake, Alberta** *Smythe, Kirsten K.[1], Colin Cooke[2], Paul Drevnick[3], Joshua Thienpont[4], Jennifer Korosi[5], Robert J. Cornett[1,6], Jules M. Blais[1,4] [1]Department of Earth & Environmental Sciences, University of Ottawa, Ottawa, ON; [2]Alberta Environmental Monitoring, Evaluation and Reporting Agency, Alberta Environment & Parks, Edmonton, AB; [3]Alberta Environmental Monitoring, Environmental Monitoring and Science Division, Alberta Environment & Parks, Calgary, AB; [4]Department of Biology, University of Ottawa, Ottawa, ON; [5]Department of Geography, York University, Toronto, ON; [6]Deceased
2:15 – 2:30	**High frequency sampling to assess the risk and temporal dynamics of pesticides in Southwestern Ontario streams** *Chemeris, Maria, Ryan Prosser School of Environmental Sciences, University of Guelph, Guelph, ON
2:30 – 2:45	**Invasive and native wetland macrophytes vary in glyphosate sensitivity and uptake** *Sesin, Verena[1], Christina M. Davy[1,2], Marcel E. Dorken[3], Janice M. Gilbert[4], Joanna R. Freeland[3] [1] Environmental and Life Sciences, Trent University, Peterborough, ON; [2] Wildlife Research and Monitoring Section, Ontario Ministry of Natural Resources and Forestry, Peterborough, ON; [3] Department of Biology, Trent University, Peterborough, ON; [4] Ontario Phragmites Working Group, Peterborough, ON
2:45 – 3:00	**Local environmental variables and metal body burden impact on the gut microbial community structures of river otter (*Lontra canadensis*) along the Athabasca River, Canada** *Guo, Galen[1], Kristin M. Eccles[1], Morgan MacMillan[1], Philippe J. Thomas[1,2], Hing Man Chan[1], Alexandre J. Poulain[1] [1]Department of Biology, University of Ottawa, Ottawa, ON; [2]Science and Technology Branch, Environment and Climate Change Canada, National Wildlife Research Center, Ottawa, ON

*** Student presentation**

Platform Presentations

Time	Session C: Multi-generational Effects Room 1130
3:45 – 4:00	**Making a case for the use of novel multi-generational and developmental ecotoxicological endpoints in freshwater gastropods** *Osborne, Rebecca K[1], Patricia L. Gillis[2], Ryan S. Prosser[1] [1]School of Environmental Sciences, University of Guelph, Guelph, ON; [2]Aquatic Contaminants Research Division, Environment and Climate Change Canada, Burlington, ON
4:00 – 4:30	**Transgenerational toxicology: the case for the widely prescribed antidepressant fluoxetine** Trudeau, V.L., M. Vera Chang, T.W. Moon Department of Biology, University of Ottawa, ON

Time	Session D: Tools for Assessing Toxicity of Environmental Contaminants Room 3105
3:45 – 4:00	**Toxicity modifying factors influence the aquatic toxicity of technology critical elements** McGeer, Jim, Rashid Shah, Katrina Medyk, Jonathan Ford, Che Lu, Scott Smith Institute for Water Science, Wilfrid Laurier University
4:00 – 4:15	**In silico genotoxicity: so which chemicals are DNA-damaging?** *Tran, Yen[1,2], Iain Lambert[1,2], Paul White[1,3], Carole Yauk[1,2] [1] Environmental Health Science and Research Bureau, Health Canada, Ottawa, ON; [2] Department of Biology, Carleton University, Ottawa, ON; [3] Department of Biology, University of Ottawa, Ottawa, ON
4:15 – 4:30	**Tackling ToxTracker® Data: The Determination of Endpoint-specific Benchmark Response to Facilitate Potency Ranking** *Boisvert, Lorrie[1,2], Paul White[1,2], Giel Hendriks[3] [1]University of Ottawa, Biology Department, Ottawa, ON; [2] Genetic Toxicology Laboratory Group, Environmental Health Science & Research Bureau, ERHSD, HECSB, Health Canada, Ottawa, ON; [3]Toxys B.V., Leiden, Netherlands

*** Student presentation**

Poster Presentations

The effects of carbaryl on a freshwater mussel (*Lampsilis siliquoidea*): A non-targeted metabolomics study

*Atkinson, Brian[1], Joe Salerno[2], Linda Lissemore[1], Dyanne Brewer[3], Ryan Prosser[4]

[1]AFL-Lab Services, University of Guelph, Guelph, ON; [2]Aquatic Contaminants Research Division, Environment and Climate Change, Burlington, ON; [3]Advanced Analysis Centre, University of Guelph, Guelph, ON; [4]School of Environmental Sciences, University of Guelph, Guelph ON

High performance liquid chromatography assay for enzymatic activity of cytochrome P4502E1 via hydroxylation of 4-nitrophenol

*Beyers, Danielle, Dr. Amy Rand

Department of Chemistry, Carleton University, Ottawa, Ontario

Impact of neonicotinoid pesticides on amphibian immunity and susceptibility to parasites

*Gavel, Melody J.[1], Sarah D. Richardson[1], Mark R. Forbes[1], Stacey A. Robinson[2]

[1]Department of Biology, Carleton University, Ottawa, ON; [2]Environment and Climate Change Canada, Ottawa ON

HPLC UV-Vis detection of a saturated fluorotelomer aldehyde (FTAL) synthesized from fluorotelomer alcohol (FTOH)

*Harris, Keegan, Dr. Amy Rand

Department of Chemistry, Carleton University

*** Student presentation**

Poster Presentations

A comprehensive evaluation of naphthalene sulfonate toxicity to aquatic biota: exposures via water, sediment, & the potential for bioaccumulation

*Matten, K.J.[1], P.L. Gillis[2], J. Toito[2], D. Milani[3], A.J. Bartlett[2], J.L. Parrott[2], V. Balakrishnan[2], R.S. Prosser[1]

[1] School of Environmental Sciences, University of Guelph, Guelph, ON; [2] Aquatic Contaminants Research Division, Environment and Climate Change Canada, Burlington, ON; [3] Watershed Hydrology and Ecology Research Division, Environment and Climate Change Canada, Burlington, ON

***In-silico* toxicity assessment of the UV transformation products of the fungicides boscalid and pyraclostrobin**

*Skanes, Blake[1], Dyanne Brewer[2], Armen Charchoglyan[2], Ryan Prosser[1]

[1]School of Environmental Science, University of Guelph, Guelph, ON; [2]Advanced Analysis Centre, University of Guelph, Guelph, ON

Come on out to Café Nostalgica after the conference for the Student Awards Social and Dinner!

601 Cumberland Street

Check out the full abstract booklet online at:

www.laurentiansetac.ca/2019-annual-general-meeting/

*** Student presentation**

Notes

Platform Presentations

Session A: Emerging Contaminants of Environmental Concern

Emerging polyfluoroalkyl substances (PFAS) metabolize to bioactive products that modify proteins and promote cell toxicity

Rand, Amy

Department of Chemistry, Carleton University, Ottawa, Ontario

E-mail: amy.rand@carleton.ca

Key Words: PFAS, fluorotelomer substances, bioactivation, toxicity

Per- and poly-fluoroalkyl substances (PFAS) have high commercial value, but given their persistence, bioaccumulation, and toxicity, there is need for new information on the fate of PFAS in biological systems, including perfluorinated carboxylic acids (PFCAs) and associated precursors and transformation products. The current study connects the biotransformation of short chain PFAS with toxicity and modes of action. While metabolism is primarily considered a detoxification pathway, leading to more polar products that are readily eliminated, a class of PFAS called fluorotelomer substances form highly persistent PFCAs and reactive fluorinated aldehydes. These fluorinated aldehydes have potential to exert oxidative stress by sequestering anti-oxidant proteins and peptides. The purpose of this study was to examine the metabolism of fluorotelomer subtances and the potential of their fluorinated aldehydes to react with biomolecules. By chemically synthesizing the fluorinated aldehydes and exposing them to amino acids (e.g. cysteine, histidine, lysine, arginine) and proteins (e.g. apomyoglobin and human serum albumin), we observed rapid binding to these model biomolecules using ^{19}F NMR and ToF-MS. *In vitro* experiments were carried out to determine the extent of protein binding from the biotransformation of fluorotelomer substances. A significant portion (~60%) of the organic fluorine in these reactions was covalently bound to the protein fraction, suggesting that protein binding may be an additional fate after exposure to fluorotelomer substances. Human liver epithelial cell toxicity was also significantly higher for the fluorinated aldehyde metabolites, with LC_{50} values two orders of magnitude less than their perfluorinated analogues (e.g. PFCAs). This work suggests a relatively unexplored consequence of exposure to PFAS, especially for those substances capable of metabolizing to bioactive products.

The effect of celecoxib on carbon tetrachloride induced liver toxicity

Harris, Todd R.[1], Sean Kodani[2], Amelia Rand[1], Jun Yang[2], Denise Imai[3], Sung Hee Hwang[2] & Bruce Hammock[2]

[1]Department of Chemistry, Carleton University, Ottawa, Ontario
[2]Department of Entomology and Nematology, University of California, Davis
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Key Words: lipids, celecoxib, carbon tetrachloride, liver fibrosis

The interaction between environmental contaminants and pharmaceuticals is of great concern, since many drugs impact pathways involved in the toxic mechanism of xenobiotics. The cyclooxygenase-2 (COX-2) selective inhibitor celecoxib is widely used in the treatment of pain and inflammation. The present study reports the effect of celecoxib in a 5-week carbon tetrachloride (CCl_4)-induced liver fibrosis mouse model. Celecoxib alone and in combination with inhibitors of the enzyme-soluble epoxide hydrolase (sEH), as well as a dual inhibitor that targets both COX-2 and sEH, were administered via osmotic minipump to mice receiving intraperitoneal injections of CCl_4 Collagen deposition was elevated in the mice treated with both celecoxib and CCl_4 compared with the control or CCl_4-only groups, as assessed by trichrome staining. Histopathology revealed more extensive fibrosis and cell death in the animals treated with both celecoxib and CCl_4 compared with all other experimental groups. Although some markers of fibrosis, such as matrix metalloprotease, were unchanged or lowered in the animals treated with both celecoxib and CCl_4, overall, hepatic fibrosis was more severe in this group. Cotreatment with celecoxib and an inhibitor of sEH or treatment with a dual inhibitor of COX-2 and sEH decreased the elevated levels of fibrotic markers observed in the group that received both celecoxib and CCl_4. Oxylipid analysis revealed that celecoxib reduced the level of prostaglandin E_2 relative to the CCl_4-only group. This indicates a direct effect of COX-2 inhibition on CCl_4-induced hepatic fibrosis.

Toxicity of novel fire suppression gels to plant germination and emergence

Graetz, Sarah, Paul Sibley, & Ryan Prosser

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E-mail: sgraetz@uoguelph.ca

Key Words: fire suppression gels, plant toxicity

Previous research has shown that fire suppression additives containing perfluorinated compounds negatively affect the environment through persistent contamination and bioaccumulation. Manufacturers have introduced 'environmentally-friendly' alternatives, but limited studies on their fate and effect in the environment have been completed. A study will be completed to investigate the toxicity of six fire suppression gels: Eco-Gel™, Thermo Gel 200L™, Fire Aid 2000™, Solberg Fire Foam™, Novacool Foam™, and F-500™.

Through direct aerial application of fire suppression gels, soil contamination can occur. Toxicity to three plant species was investigated through a root elongation assay and a seedling emergence test. The crop species *Fagopyrum esculentum* (buckwheat) and *Raphanus raphanistrum* ssp. *sativus* (radish), were tested in addition to the flowering plant species, *Rudbeckia hirta,* as it is commonly found within the boreal forest where fire suppression gels may be used to control wildfires.

It was found that there was a large variation in toxicity between the fire suppression gels tested, and that some would pose a hazard to plants when released into the terrestrial environment. With the knowledge gained from this study, a better understanding of the potential effects of fire suppression gels on terrestrial ecosystems can be developed.

Identification of targets of aryl phosphate flame retardant toxicity in the rat liver, ovary and adrenal glands

Michael Wade[1], Alice Kawata[1], Marybeth Creskey[2], Terry Cyr[2]

[1] Environmental Health Science & Research Bureau, Health Canada.
[2]Centre for Biologics Evaluation, Biologics and Genetic Therapies, Health Canada

Exposure of rats to phosphate flame retardant (isopropylated triphenyl phosphate IPTPP) caused liver, adrenal gland and ovary enlargement. To understand the consequences and potential human relevance of these effects, we sought to identify the target molecule(s) mediating IPTPP toxicity in these organs. IPTPP was hypothesized to induce toxicity, like other organophosphates, by reacting with and inhibiting (a) serine hydrolase enzyme(s) (SHE). Liver, adrenal glands and ovaries were collected from female rats after oral exposure to corn oil (control) or 100 mg/kg IPTPP. Tissue homogenates were reacted with a fluorophosphate bait molecule (FP) that irreversibly binds to the active site of any SHE. Initially, FP coupled with AlexaFluor 488 was used and resulting samples separated by PAGE. A difference in fluorescently labelled proteins in liver, adrenal and ovarian between control and treated samples indicated that SHEs were pre-reacted and inhibited by IPTPP. Reacted SHEs in homogenates incubated with desthiobiotin-labelled FP were concentrated with streptavidin beads. Proteins eluted after concentration were identified using LC-MS. SHE Proteins identified in control samples but underrepresented in IPTPP-treated samples were Carboxyesterase 1E (CES1E) and monoacylglycerol Lipase ABHD6 in liver and Hormone Sensitive Lipase (HSL) in both ovary and adrenal glands. IPTPP inhibited rat liver CES and the human homolog of rat CES1E (CES1) activity but the effect on the latter enzyme was slightly less (IC50 of 320 nM vs 770 nM, respectively). Studies of HSL inhibition by IPTPP and metabolites are ongoing. These studies have identified molecular targets for aryl phosphates and may provide the basis for rapid assays to compare potency and potential hazard across the diverse class of organophosphate flame retardants.

Session B: Field Impacts of Toxicants in Aquatic Systems

Source Determination of Polycyclic Aromatic Compounds for In-Situ Oil Sands Operations in Cold Lake, Alberta

Smythe, Kirsten K.[1], Colin Cooke[2], Paul Drevnick[3], JoshuaThienpont[4], Jennifer Korosi[5], Robert J. Cornett[1,6], Jules M. Blais[1,4]

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[2]Alberta Environmental Monitoring, Evaluation and Reporting Agency, Alberta Environment & Parks, Edmonton, AB
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Key Words: PACs, in-Situ, oil sands, Cold Lake

Most of the published environmental research on Canada's oil sands concentrates on open-pit mines and bitumen processing operations. Less is known about environmental impacts of in-situ operations, like those of the Cold Lake deposit in Central-Eastern Alberta, using steam injection to extract bitumen from deep below surface. In 2012, in-situ operations surpassed open-pit mining as the dominant method of oil sands extraction in Canada, yet limited research on contamination attributed to this method is available. Here we examine polycyclic aromatic compound (PAC) profiles in dated sediment cores from 11 lakes in the Cold Lake area to track changes in PAC deposition between preindustrial and present levels. We use alkylated PACs, predominantly petrogenic contaminants, to evaluate in-situ operations as potential contamination sources to surrounding lakes. We predict that similarly to open-pit operations, concentrations of alkylated PACs in lakes sediments will increase with industrial activity and will correspond to proximity from in-situ operations. Preliminary trends demonstrate increasing concentrations of alkylated PACs at the onset industrial activity in in-situ surrounded lakes, however statistics are required to confirm. With >80% of Canadian bitumen reserves requiring in-situ techniques for extraction, this study addresses contamination attributed to these operations and highlights the importance of ongoing monitoring.

High frequency sampling to assess the risk and temporal dynamics of pesticides in Southwestern Ontario streams

Chemeris, Maria, Prosser, Ryan

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Key Words: exposure characterization, temporal dynamics

Since 1964 the Provincial (Stream) Water Quality Monitoring Network (PWQMN) program had been measuring and tracking changes of water quality in rivers and streams across Ontario. However, the network monitors only persistent organic pollutant and current use pesticides are not part of this program. A pesticide monitoring program coordinated by the OMECP and OMAFRA monitors current-use pesticides by taking 4-10 samples per year from 18 locations across the province of Ontario. Therefore, due to the lack of field data on pesticides in streams during flow events, the temporal dynamics of the concentration of pesticides in Southwestern Ontario remain poorly characterized.

To generate an alternative approach for monitoring pesticides and to increase our understanding on how pesticide exposure changes during flow events, autosamplers were deployed at three different sites in the Kettle Creek Watershed and were programmed to collect water samples every hour over the growing season (July to October) in 2018. Samples for analysis were then selected based on rainfall and flow data at the sampling sites to increase the cost-effectiveness of this approach. The data showed that after significant rain and flow events, higher concentrations of pesticides where detected. This helps us to understand the baseline and peak concentrations of pesticides that aquatic systems can experience along with the frequency and duration of these peaks. Also, in order to determine if there is any correlation between the pesticide detected and the crop type used, the land use upstream of the catchment was quantified and compared to the pesticides detected, making this approach a possible predictive tool in exposure characterization.

Invasive and native wetland macrophytes vary in glyphosate sensitivity and uptake

Sesin, Verena[1], Christina M. Davy[1,2], Marcel E. Dorken[3], Janice M. Gilbert[4] & Joanna R. Freeland[3]

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[2] Wildlife Research and Monitoring Section, Ontario Ministry of Natural Resources and Forestry, Peterborough, ON
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Key Words: bioaccumulation, herbicide, microcosm, toxicity

Invasive aquatic plants can disrupt native wetland communities, with considerable ecological and economic impacts. Glyphosate-based herbicides can control invasive plants, thereby potentially restoring native plant biodiversity, but glyphosate is toxic to all plants. Land managers therefore must weigh the benefits of using glyphosate to manage invasive plants against the potential harm to nearby non-target plants. A balanced decision requires understanding of the individual sensitivities of target and non-target plants, and the persistence of glyphosate in plant tissues post-exposure. We performed an experimental outdoor microcosm concentration-response study, in which we sprayed seven different glyphosate concentrations (Roundup WeatherMAX® formulation) representing 'real-world' applications on two emergent plants that have invaded North American wetlands, *Phragmites australis* and *Typha x glauca*, and a native co-occurring plant, *Typha latifolia*. At 27 days post-exposure we compared sensitivity and accumulation of glyphosate, AMPA (one degradation product) and alcohol ethoxylate surfactant (from formulation) among the three taxa. *Phragmites australis* exhibited higher glyphosate sensitivity than both *Typha* taxa and accumulated more glyphosate and AMPA in above-ground tissues. We observed a qualitative trend of lower sensitivity and lower glyphosate and AMPA accumulation in *T. x glauca* compared to *T. latifolia*. Accumulation of surfactant residues was similar among taxa. This is the first evidence of inter- and intra-generic differences in glyphosate sensitivity and accumulation in emergent macrophytes. Our data show that responses of emergent macrophytes to glyphosate cannot be generalized among taxa. Understanding this variation can improve accuracy of predicted responses of emergent wetland plants to glyphosate. These predictions can contribute to implementing effective and sustainable wetland management plans. Our detected glyphosate retention in treated plant tissues calls for further research on its fate in macrophytes.

Local environmental variables and metal body burden impact on the gut microbial community structures of river otter (*Lontra canadensis*) along the Athabasca River, Canada

Galen Guo[1], Kristin M. Eccles[1], Morgan MacMillan[1], Philippe J. Thomas[1,2], Hing Man Chan[1], Alexandre J. Poulain[1]

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Keyword: gut microbiome, metagenomic, river otter, biomarker

The Athabasca region is home to one of the largest oil bitumen deposit in the world. One of the mandates of Environment and Climate Change Canada (ECCC) is to monitor wildlife fitness status including those residing in proximity to the oil sands in the Athabasca region. ECCC's current project aims to evaluate the impact of contaminant released from the oil sand in the Athabasca region on river otters (*Lontra canadensis*) health. As a result of its sensitivity to disturbances and small home range, river otters are designated as a sentinel species of freshwater ecosystems. The use of these sentinel species is currently often destructive and invasive and could be detrimental to current protection and conservation effort. The goal of this study is to determine whether changes in river otter gut microbiota community structures in response to Athabasca oil sand activities, could be used as a biomarker for anthropogenic disturbances. To address this question, we obtain trapped river otter surrounding the surface mining activity in the Athabasca Region. We used high-throughput sequencing of the 16S rRNA V3-V4 region to evaluate the gut microbiome of river otter intestine. We identified four distinct enterotypes in the region and identified environmental variables driving those changes. We also show that oil sands mining has no significant impact on river otter gut microbiota. However, anthropogenic disturbances in the area are severe and could overshadow any potential effect on the gut microbiome. Further investigations into existing active pipelines and oil pumps are required. Developing a better understanding between the dynamic of the gut microbiota and its host habitat could benefit efforts in conservation and protecting wildlife and study ecosystem health.

Session C: Multi-generational effects

Making a case for the use of novel multi-generational and developmental ecotoxicological endpoints in freshwater gastropods

Osborne, Rebecca K[1], Patricia L. Gillis[2], and Ryan S. Prosser[1]

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Key Words: method development, aquatic ecotoxicology, developmental biology, freshwater molluscs

Gastropods play a critical role in aquatic ecosystems and often make-up a significant proportion of the benthic biomass. Despite their considerable diversity and importance, freshwater gastropods are severely underrepresented in ecotoxicological studies and account for fewer than 1% of the publications in the US Environmental Protection Agency's ECOTOX database. To evaluate the potential utility of multi-generational contaminant studies and develop feasible generational test designs, as well as to characterize the normal growth and development of the freshwater file ramshorn snail (Planorbella pilsbryi), we conducted both a multi-generational toxicity test and several snail embryo development studies. During the multi-generational study, adult exposure to five sub-lethal concentrations of copper resulted in decreased reproduction with increasing copper exposure and complete reproductive inhibition at the highest concentration (75 µg/L). Mortality and inhibition of reproduction were not observed in the control and three lowest concentrations (4.69, 9.38, 18.75 µg/L Cu) over the course of the exposure and during recovery in clean water indicating no lasting adverse effects. However, subsequent exposure of the unexposed juveniles that were produced during the recovery period (i.e. those not directly exposed to copper) showed that juveniles born to copper-exposed parents (LC50: 11.57 µg/L Cu; 95% CI: 3.71-19.43 µg/L Cu) were significantly less tolerant to copper exposure than juveniles born to unexposed parents (LC50: 29.25 µg/L Cu; 95% CI: 22.17-36.32 µg/L Cu). Despite no obvious changes in parental reproductive success, the fitness of un-exposed juveniles was compromised due to parental exposure. Further studies to characterize development in P. pilsbryi embryos and to evaluate the potential use of developmental endpoints as a sensitive and non-invasive bioindicator in environmental risk assessment and freshwater mollusk conservation are ongoing.

Transgenerational toxicology:

the case for the widely prescribed antidepressant fluoxetine

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Key Words: Prozac, fish, epigenetic, stress, behaviour

Antidepressants are widely prescribed to many patients including pregnant or breast-feeding mothers. Risks to offspring and descendants are beginning to be assessed in humans, but long-term studies will require many decades of analysis. Moreover, a multitude of antidepressants are widely detected in aquatic ecosystems, and environmental levels are biologically active in some fish. Small bodied, with a relatively short generation time, the zebrafish *(Danio rerio)* represents an amendable model for transgenerational toxicology. Data will be presented the demonstrate that short early-life exposures to fluoxetine, the active chemical in Prozac disrupts the stress axis (cortisol synthesis and associated transcriptional signatures in the kidney-adrenal complex) and exploratory behaviours (novel tank test) across multiple generations. Time- and sex-dependent effects are evident. Current data implicate epigenetic inheritance of a hypocortisol phenotype linked to significantly reduced exploratory behaviour across 3 generations. Both human and ecosystem-related health implications will be discussed.

Session D: Tools for Assessing Toxicity of Environmental Contaminants

Toxicity modifying factors influence the aquatic toxicity of technology critical elements.

McGeer Jim, Shah Rashid, Medyk Katrina, Ford Jonathan, Lu Che, Smith Scott.

Institute for Water Science, Wilfrid Laurier Univeristy

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Key words: metals, rare earth elements, toxicity, mixtures.

The growing use of rare earth elements and other technology critical element such as In, Ge and Ga in personal electronic devices, green technologies and medical applications results in a developing concern for impacts in aquatic environments. However, there are no water quality guidelines/criteria for TCEs and few studies available. The objective of this research is to contribute data towards the establishment of assessment tools for the effects of TCEs. We have studied the toxicity of Ce, Sm, Dy Tm, In, Ge and Ga to sensitive invertebrates (*Hyalella azteca* and *Daphnia*). The toxicity modifying influences of cationic competition (Ca, Mg and Na) and dissolved organic matter (DOM) were assessed. With some exceptions, Ca and DOM provide protection against toxicity but the incorporation of these effects into toxicity prediction models was inhibited by a lack of understanding of solution geochemistry. Funded by NSERC and Environment Canada via a Strategic Grant.

In silico genotoxicity: so which chemicals are DNA-damaging?

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Key Words: anthraquinones, genotoxicity, predictive modelling, hazard assessment

A vast number of chemicals are present in the Canadian environment and have not been tested for toxicity (i.e., data-poor). Genotoxicity (i.e., DNA damage) assessment is imperative to reduce risk of genetic diseases and cancer. Quantitative structure-activity relationship models (i.e., (Q)SAR) provides cost-and-time-efficient means to identify genotoxicity among data-poor chemicals. Based on chemical structures and mathematical algorithms, (Q)SAR predicts the chemicals' genotoxic effects.

The study's overarching objective was to apply *in silico* (Q)SAR and *in vitro* assessments to determine potential genotoxic hazard among a large list of data-poor environmental chemicals.

Tackling ToxTracker® Data: The Determination of Endpoint-specific Benchmark Response to Facilitate Potency Ranking

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Key Words: ToxTracker, genotoxicity, data analysis, Benchmark Dose

Toxys B.V. developed an assay called ToxTracker, which detects genotoxicity by monitoring six reporter genes in cultured mouse cells. The reporters encode signal proteins that respond to chemically-induced genetic damage, and are detected by fluorescence as a measure for DNA damage response. The ToxTracker assay generates large amounts of complex, multivariate dose-response data, creating a need to devise appropriate data processing techniques that facilitate rapid and effective interpretation of assay results.

This study involved the use of novel strategies to develop a data analysis and interpretation strategy to efficiently scrutinise large amounts of ToxTracker dose-response data. More specifically, the work is (i) defining a threshold for identification of a significant positive response, (ii) defining endpoint-specific critical effect sizes by using the 95th percentile of the log-normal distribution in control responses; and (iii) using the Benchmark dose covariate approach to effectively rank validation compounds by potency and (iv) investigating the statistical relationships between reporters.

The results obtained define a threshold of 1.5-fold change for identification of a positive response, which is consistant with the value advocated by Toxys B.V. A pipeline for potency ranking for rapid interpretation of data for new test articles was also developed. It readily permits potency evaluations for responses of each of the six reporters. A principal component analysis revealed that the reporters for genotoxicity are not completely independent of each other.

Overall, the results obtained support the development of a strategy for efficient quantitative analyses of ToxTracker assay dose-response data. Next steps include a machine learning algorithm to classify compounds based on their dose-response profile.

This study contributed to the continuing development of robust data analysis strategies to facilitate effective use of quantitative dose-response data in order to screen genotoxic chemicals. More specifically, it contributes toward validation of the assay for intcorporation into regulatory frameworks.

Poster Presentations

HPLC UV-Vis Detection of a Saturated Fluorotelomer Aldehyde (FTAL) Synthesized from Fluorotelomer Alcohol (FTOH)

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Key words: per and poly-fluoroalkyl substances, fluorotelomer alcohols, metabolism, HPLC

Per- and poly-fluoroalkyl substances (PFAS) are highly fluorinated aliphatic substances that have two or more carbon atoms. PFAS have unique characteristics compared to their hydrocarbon analogs that make them useful in a wide range of industrial and commercial applications, such as in textiles and leather, paper and food packaging, fire-fighting foam, and cosmetics. Concern has been raised about PFAS because of their environmental persistence, global occurrence, and presence in human blood. One class of PFASs are the fluorotelomer alcohols (FTOHs), which humans are exposed to through indoor environments and commercial products. Recent evidence has shown that FTOHs biotransform to an electrophilic fluorotelomer aldehyde (FTAL) metabolite that may be toxic to cells through covalent protein binding.

The purpose of this project was to chemically synthesize FTAL, which is expensive and difficult to obtain, and develop a detection method using HPLC-UV/Vis. Under an inert atmosphere, FTOH was oxidized to FTAL using the reagent Dess Martin periodinane (DMP). The mixture was extracted and the solvent was removed under vacuum. Confirmation of FTAL synthesis was obtained through the comparison of experimental to literature ^{19}F and ^{1}H NMR spectroscopy data. The synthesis reaction was optimized to achieve an 80-90% by weight solution of FTAL. Derivatization of FTAL using 2,4-dinitrophenylhydrazine (2,4-DNPH) was necessary for HPLC-UV/Vis detection. The derivatized FTAL was identified by reversed phase HPLC using a water/acetonitrile isocratic elution, detected at 365 nm. This technique can be used in future research to further elucidate FTOH metabolism in biological systems by quantifying FTAL metabolites. The synthesized FTAL standard can also be dosed in cell and animal models to determine its mechanism of cytotoxicity.

High performance liquid chromatography assay for enzymatic activity of Cytochrome P4502E1 via hydroxylation of 4-nitrophenol

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Key words: PFAS, CYP2E1, enzyme activity

The enzyme cytochrome P450 2E1 (CYP2E1) plays an important role converting toxicants into polar metabolites that can be eliminated from the body. However, CYP2E1 also transforms substrates into electrophilic aldehyde metabolites, which can lead to cell toxicity through protein covalent binding. The purpose of this study was to develop an assay to measure CYP2E1 activity to quantify its role in the metabolism of emerging toxicants. Specifically, we studied the hydroxylation of p-nitrophenol (PNP), a selective CYP2E1 substrate, to p-nitrocatechol (PNC) to measure CYP2E1 *in vitro* activity in rat liver microsomes. We used both UV-vis spectroscopy and HPLC to quantify levels of PNC formed when PNP is metabolized by CYP2E1. Initial results gained from UV-vis spectroscopy were inadequate, resulting from sensitivity limitations. We therefore developed and optimized an HPLC-UV method to quantify the formation of PNC in microsomes. PNP (100 µM) was incubated in microsomes for 5 min, after which the reaction was stopped using ice cold acetonitrile (ACN). The internal standard salicyl amide was added, and the suspension was centrifuged and filtered prior to HPLC analysis. The PNC formed from PNP metabolism by CYP2E1 was quantified by peak area comparisons to PNC standards (5 - 30 µM, R^2 = 0.99). The PNC mean concentration equaled 1.47 $\pm$ 0.22 µM, giving an average enzymatic activity of 0.21 $\pm$ 0.06 µmol/min·mg (n = 3). Using the same microsomal system, we are currently examining the contribution of active CYP2E1 to the metabolism of polyfluoroalkyl substances (PFAS). With a short reaction incubation and chromatographic run time (7 min) the assay is reproducible and may be applied to other systems (e.g. tissue or cell extracts) to understand the contribution of CYP2E1 in toxicant metabolism.

In-silico toxicity assessment of the UV transformation products of the fungicides boscalid and pyraclostrobin

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Key Words: fungicides, *in-silico* toxicology, mass spectroscopy, oxidation

The use of oxidative processes to control biological contamination of food is on the rise. Ultra-violet (UV) light, among other methods of oxidation, has been shown to be effective at limiting bacteria and fungal growth on produce. While this is beneficial to producers and consumers alike through increased shelf life, consideration must be given to the potential transformation of organic compounds, like pesticide residues, that are not the intended target of the oxidation treatment. Boscalid and pyraclostrobin, the active ingredients in the commercial pesticide Pristine®, are fungicides that see regular agricultural use. The fungicides were independently exposed to a variety of doses of UV light. Degradation rate was observed through LC-MS. Samples showing considerable degradation of the parent fungicide were further investigated using non-targeted q-ToF analysis. Using molecular formula, exact mass and spectral analysis, between the obtained chromatographs and the literature, putative structures for transformation products of these compounds were identified. Using *in-silico* toxicology through the EPA's T.E.S.T. and EU's Toxtree programs toxicity of the putative transformation products was compared to the parent fungicide. Whereas the parent fungicides have undergone extensive toxicity testing in development, very little is currently known about the toxicity and, thus, potential hazard to consumers posed by their transformation products.

The effects of carbaryl on a freshwater mussel (*Lampsilis siliquoidea*): A non-targeted metabolomics study

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Key Words: biomarker, hemolymph, metabolomics, non-lethal, pesticides, Unionidae

Acute and chronic exposure to agricultural pesticides poses concerns for populations of aquatic organisms. There is a need to investigate new screening tools for describing and measuring organism response in toxicological studies. These tools need to be able to assess stress sub-lethally, be cost effective, and reproducible. An analysis of circulatory fluid (hemolymph) from freshwater mussels offers a non-lethal sampling alternative that is simple to conduct, non-intrusive and provides reliable data. Metabolomics is a technique that provides insight into biochemical pathways being affected in response to the toxic stressor.

This research evaluated the effects of a 28-day exposure of Carbaryl on the metabolome of the freshwater mussel *Lampsilis siliquoidea* (fatmucket). Carbaryl is a Carbamate pesticide commonly used in agriculture to control insects. After exposure significant changes were found on 16 metabolites at both treatment levels. Specifically found was alpha-N-phenylacetyl-L-glutamine, which has strong indications of the organism's response to uremic conditions and kidney dysfunction. These results suggest that examining hemolymph as a non-lethal sampling technique through a metabolomics study provides value added information for assessing risk and toxicity.

A comprehensive evaluation of naphthalene sulfonate toxicity to aquatic biota: exposures via water, sediment, & the potential for bioaccumulation

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Keywords: aquatic toxicology, benthos, bioavailability, risk assessment

Naphthalene sulfonic acids (NSAs) are used extensively in Canadian industries (e.g., dispersant in dyes, rubbers, pesticides or anti-corrosive agent in coatings, sealants) despite the gap in our knowledge of how they may affect aquatic biota upon their introduction to the environment. This project examined the toxicity of three priority NSA congeners; dinonylnaphthalene disulfonic acid (DNDS), barium dinonylnaphthalene sulfonate (BaDNS), and calcium dinonylnaphthalene sulfonate (CaDNS) to a range of freshwater biota. These compounds primarily partition into sediments in aquatic environments and desorb into water with time. Part 1 investigated the impact of NSAs on the hatchability and maturation of the pelagic *Promelas pimephales*, and acute toxicity to epibenthic invertebrates exposed only through water. Part 2 investigated NSA toxicity to benthic invertebrates *Hyalella azteca* and *Tubifex tubifex* and how substrate composition impacts toxicity. Chronic effect characterization was done by exposing organisms via spiked substrates to emulate relevant environmental exposure routes, as these chemicals have a relatively large affinity for the organic carbon of sediment (log Koc). Substrate containing more organic carbon had a protective effect for both organisms; 28-d LC50s for juvenile *H. azteca* in sand were >500, 113, and 69 µg/g dry weight (dw), and in sediment were >2000, 832, and 648 µg/g dw, for DNDS, BaDNS, and CaDNS, respectively. Similarly, 28-d EC50s for juvenile production of *T. tubifex* in sand were 638, <20, and <20 µg/g dw, while in sediment were 2336, 398, and 205 µg/g dw, for DNDS, BaDNS, and CaDNS, respectively. Part 3 investigated the bioaccumulative tendency of NSAs in the freshwater mussel *Lampsilis siliquoidea* and oligochaete *T. tubifex*. Both tests followed OECD guidelines and offer insight into NSA movement throughout the environment. This study will support the eventual aquatic risk assessment of this group of chemicals in Canada, covering a wide range of species and exposure pathways.

Impact of neonicotinoid pesticides on amphibian immunity and susceptibility to parasites

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Key Words: amphibian, neonicotinoids, parasites, immune systems

Neonicotinoids are a widely-used class of insecticides registered for use in over 120 countries. These pesticides are commonly found in agricultural waterways, used by amphibians for breeding. Though neonicotinoids are regarded as generally safe to vertebrate organisms, previous research has found that pesticides can negatively impact amphibian immunity and their susceptibility to disease. This study looks at the effects of chronic exposure to neonicotinoids on blood cell profiles and stress-hormone concentrations of wood frogs (*Lithobates sylvaticus*). Furthermore, it looks at the impacts of two-week exposure to neonicotinoids on leopard frog (*Lithobates pipiens*) immune systems and susceptibility to *Echinostoma* spp. parasites. Finally, this study examines the impacts of neonicotinoids on *Echinostoma* spp. parasite survival to ascertain a net effect. Exposure to neonicotinoids was found to cause anemia and induced a state of moderate stress in wood frogs, and also impacted their stress-response. However, neonicotinoids did not impact leopard frog immune systems or their susceptibility to parasites; nor did they impact the survival of parasites. Overall, these results suggest that exposure to neonicotinoids can have a negative impact on amphibian physiology, but that these effects may be species-specific or exposure-time specific.